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# MUTATIONS IN AGRICULTURAL PLANTS

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## INTRODUCTION.

THE pioneer work of STADLER on induced mutations is fundamental also to plant-breeding. Yet STADLER takes up an almost negative position in regard to their practical value. »But rare favourable mutation is likely to be accompanied by unfavourable mutations induced by the same treatment (1930, p. 18) . . . The result is a plant heterozygous for several genes, mostly undesirable.» (Even so late as 1943 a similar conception, though not so pronounced a one, was advanced by BURGEFF.) STADLER continues: »There is little chance of producing experimentally variations which have not already occurred in nature». He is however more optimistic as regards the breeding of fruit trees. These are vegetatively propagated; by means of induced mutations a new variation is directly created and gamete formation and fertilization are avoided.

American workers do not seem to have arrived at any positive results or conclusions, at least so far as agricultural plants are concerned (LINDSTROM, 1933; MAC ARTHUR, 1934; GENTER and BROWN, 1941). GOODSPEED and co-workers (1929, 1934, etc.), who induced and analysed numerous chromosome abnormalities in *Nicotiana*, have not continued their investigations in this field.

Several Russian investigators have been considerably more positive in their attitude, first and foremost DELAUNAY and SAPEHIN, who in a series of papers described vital mutants in wheat, several of which possess practical interest. The former published in 1934 a synoptical work which, according to a review in Resumptio Genetica, 10, p. 164, stresses the value of induced mutants for plant-breeding. Yield tests do not seem to have been carried out. In any case no crop figures have been published.

German research-workers began their experiments with induced mutations at an early time. To begin with, however, their works were more of a theoretical character. First in the years 1942 and 1943 FREISLEBEN and LEIN described extensive model experiments in barley. After X-ray radiation and mass infection they even succeeded in pro-

ducing a mildew-resistant line. Much earlier, SENGBUSCH and HACKBARTH had shown that spontaneous mutants can successfully change a species and thereby improve its agricultural value (in their case sweet lupine).

The German works on induced vital mutants were first undertaken on a large scale after a lecture held by NILSSON-EHLE in the year 1939 in Halle on polymeric factors in barley. Unfortunately, this lecture was only published in the form of a short notice. As early as the mid-thirties he produced vital mutants (p. 16), at least one of which was found to be equal to the mother-line in yield. Crosses with the very straw-stiff malt-barley line Kenia have given, according to a personal communication, products with still higher straw-strength and, simultaneously, very good productivity.

The experiments which underlie the present work, and which have further shown the practical value of the induced mutants, were commenced early (1929—30) and have been promoted in various ways by NILSSON-EHLE. Since January, 1940, the cost of all the work on induced mutations has been defrayed from funds donated by A.B. Saltsjöqvarn, Stockholm, and A. B. Mårten Pehrsons Valsqvarn, Kristianstad, through the medium of the Head of the Swedish Seed Association, Professor Ä. ÅKERMAN. This has rendered it possible to extend the experiments considerably. The practical work is being conducted in the different departments of the Swedish Seed Association. Mutants in barley, wheat, oats and flax appear to be especially promising from a practical point of view. But also in soybeans, sweet lupine and oleiferous species of the Cruciferae mutants of interest have been observed. Here as elsewhere results are obtained first after lengthy and extensive experiments.

Certain analyses for this work have been carried out by Statens Centrala Frökontrollanstalt (the State Seed Testing Station) (Professor G. NILSSON-LEISSNER) as well as by A.B. Stockholms Bryggerier (H. THUNÆUS, Engineer). The heads of the branch stations of the Seed Association have carried out special yield trials.

Of recent years the irradiation experiments have been conducted by the Radiophysics Institute, Stockholm (Director: Professor R. SIEVERT), under the supervision of Dr. A. FORSSBERG.

In the account of the work submitted here theoretical and practical results have been interwoven. In spite of its size this paper gives merely a *survey* of the results rather than an *exhaustive account*. The physical

and mathematic problems, related to X-ray irradiation, are discussed in the work of LEA (1946), to which the reader is referred.

## I. EFFECT OF X-RAY RADIATION IN THE $X_1$ GENERATION.

According to the definition applied in this paper plants raised from X-rayed seeds form an $X_1$ generation. The $X_1$ plants are often heterozygous for induced genome changes of various kinds. After self-fertilization they give rise to an $X_2$ generation. In this or in later generations ($X_3$, $X_4$) mutants segregate out. Mutations can also be induced by irradiation of pollen-grains and egg-cells. This method is more circumstantial and is not particularly suitable for practical purposes.

### 1. COURSE OF MITOSIS IN IRRADIATED SEEDS.

X-rayed dry seeds germinate even when they have received very high X-ray doses. In *Avena*, for instance, the radicle and the rootlets take up water, pierce the coleorhiza and after 75.000 *r* still show nuclear divisions, although these run an extremely irregular course. However much the interphase chromosomes of the resting seeds may have been affected or fragmented, the cells can nevertheless begin their prophase, complete metaphase and anaphase and enter telophase and interphase stages. After that, however, the most damaged cell-nuclei degenerate or become inactivated, so that after a few mitotic cycles the divisions have an almost normal appearance. Interphase chromosomes may thus be broken into pieces without this influencing the immediate capacity for division to any notable extent.

Irradiation of resting seeds causes solely the so-called secondary effect, i. e. the chromosomes are fragmented and translocated (or inverted) in the numerous breaks that have arisen. Irradiation of nuclear divisions while in progress, on the other hand, is followed by *the primary effect* with its irregular fragmentation and agglutination of chromosomes (»stickiness»), *the mitose-free intermediate period* when division ceases, and *the secondary effect*, which is observed in those cells which were in interphase stage on being rayed. The intenser the irradiation has been, the longer will the mitose-free period be. As dry seeds contain only resting nuclei, it is evident that they can show nothing but the secondary effect. From the works recently published by EULER, HEVESY and their associates (1944, 1945) we know that the mitose-free period occurs because desoxyribose nucleotides are not formed. A certain quantity of nucleotides must be present for mitoses to start.

GUSTAFSSON (1937), GELIN (1941) as well as FRÖIER, GELIN and GUSTAFSSON (1941) have reported results concerning the frequency of

disturbances in irradiated seeds. GELIN observed in Golden barley (Gullkorn), treated with 10.000 *r*-units, 13 % and 28 % disturbed anaphases at the first division of the cells, the lower frequency in seeds having a water content of 10 %, the higher in seeds with a water content of 15 %. FRÖIER, GELIN and GUSTAFSSON found that an identical dosage was followed by fewer disturbances in diploid species than in tetraploids, and fewer in the latter than in hexaploids, and that the number of disturbances induced by low doses is proportional to the number of genomes (cf. also SMITH, 1943). A dosage of 5.000 *r* gave 11 % disturbed cells in *Triticum monococcum* (2n = 14), on an average 22 % in *T. durum* and *dicoccum* (2n = 28), and 29 % in *T. vulgare* (2n = 42). Reduced to the diploid level (2n = 14) the figures will be 11,0—11,1—9,8 %. The *immediate* frequency of disturbances is already rather high at this dose. At 25.000 *r* it amounted to 99 % in *T. durum*, 96 % in *T. vulgare* and 96 % in *Avena sativa*. Thus, after this X-ray dose practically all observed cell divisions were badly damaged.

If the seeds have been steeped in water before irradiation, or their water content is raised in some other way, the mitotic disturbances also increase in number. This also takes place if the seeds are drenched with metallic salts or pretreated with various cell-poisons. In a certain experimental series with Golden barley GELIN (1941) obtained disturbance frequencies of 13, 28, 54 and 51 % respectively in seeds containing 10 % $H_2O$, 15 % $H_2O$, or that had been steeped in water or heteroauxin for 24 hours. Pretreatment of this kind also raises the mutation frequency.

The extent to which the external environmental conditions can influence the disturbance effect has been clarified by GELIN in a series of practically important but as yet unpublished investigations. Among other things he has shown that chromosome disturbances increase substantially in number if the temperature falls towards zero at germination, but that they decrease if the temperature is raised. The same result has been obtained by SAX (1939) after irradiation of pollen-grains of *Tradescantia*.

## 2. SEEDLING LETHALITY, SPROUTING ABILITY AND $X_1$ STERILITY.

For all mutation work it is of importance to fix suitable irradiation doses. The X-ray effect can be determined either by means of laboratory tests of germination and sprouting ability, the dose being settled that reduces the sprouting ability of the $X_1$ plants to a suitable degree, or by field experiments in which the number of plants harvested after differ-

ent X-ray doses is calculated. These two testing methods do not give the same result because field material is always exposed to considerably harder conditions. Only field tests give a fully reliable verdict. For preliminary investigations or for work of a special nature laboratory tests will suffice.

Different kinds of plants react very dissimilarly to X-radiation (GUSTAFSSON, 1944). Especially insensitive are the seeds of the Cruciferae, which in certain cases (white mustard and swedes) stand doses of wellnigh 100.000 *r* and even more. On the other hand, the seeds of the pea plants are very sensitive, their maximum dose being only 10.000—20.000 *r*. The same applies to the seeds of hemp, sunflowers and safflowers, which cannot endure 10.000 *r*. For practical work we have tried to fix what may be called the »critical dose», i. e. the dose that while still producing a sufficiently large field material induces a maximum of mutations and chromosome re-arrangements. The »critical dose» falls with increasing water content of the seeds.

Types of plants having seeds that can stand high X-ray irradiation are also rich in fatty acids (oil). Naturally this does not mean that the oil content alone determines the difference in susceptibility. Rather is this a phenomenon that depends partly on the family to which the plant belongs, partly on the general chemical properties of the seeds, including the oil content and water content.

For the common cereals wheat, oats, barley and rye an X-ray dose of 10.000—20.000 *r* is the most suitable. The effect varies however considerably in different years. For winter wheat and winter rye this is quite natural, as the injured seedlings have to bear the cold of winter. In spring barley, spring wheat and oats the result also varies with the nature of the soil and the climatic conditions during the period of vegetation.

A special method, particularly applicable to species with ray-resistant seeds, consists in having the irradiated seeds stored for one or more years before they are sown. This procedure has been tried with advantage in white mustard, swedes and rape. The long storage has the result that the X-ray effect is substantially intensified.

In proportion as the X-raying increases in intensity various inhibitions of development make their appearance. These manifest themselves in such manner that the sprouting capacity falls, the first leaves become yellow and shrivelled, often the sprout does not pierce the surface of the soil, or, if this is the case, it dies at an early stage, etc. (The yellowing of the leaves does not solely depend upon the genomatic dis-

turbances but is also connected with the inactivation or destruction of the plastids, see e.g., HRUBY, 1935.) The plants that survive develop more or less sterile inflorescenses and fruits depending on the quantity of irradiation, and are now and then abnormal in other respects (ramified, stunted, lacking chlorophyll). Photographs illustrating the X-ray effects can be found in FREISLEBEN and LEIN (1943) and GUSTAFSSON (1944).

The relation between the fertility of the $X_1$ plants and the X-ray dose given has been determined in two extensive series of two-rowed

TABLE 1. *X-ray dosage and $X_1$ fertility.*

| | Maja barley | | | | Ymer barley, line 40/13 $b_7$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Per cent harvested plants | Average fertility % | Per cent normally fertile plants | N | Per cent harvested plants | Average fertility % | Per cent normally fertile plants | N |
| Control ..... | 75 | 97,1 | 98 | 90 | ? | 96,1 | 100 | 25 |
| 500 *r* ..... | — | — | — | — | 51 | 94,2 | 92 | 50 |
| 1.000 *r* ..... | — | — | — | — | 61 | 92,8 | 80 | 50 |
| 2.500 *r* ..... | 64 | 89,5 | 63 | 557 | 57 | 91,8 | 74 | 50 |
| 5.000 *r* ..... | 32 | 77,6 | 30 | 277 | 42 | 82,0 | 36 | 50 |
| 7.500 *r* ..... | — | — | — | — | 44 | 75,2 | 22 | 50 |
| 10.000 *r* ..... | 16 | 75,4 | 14 | 142 | 41 | 75,6 | 12 | 203 |
| 15.000 *r* ..... | 13 | 73,8 | 10 | 91 | 39 | 68,7 | 10 | 196 |
| 20.000 *r* ..... | 10 | 74,1 | 9 | 140 | 37 | 61,9 | 2 | 185 |
| 25.000 *r* ..... | 8 | 66,5 | 4 | 119 | 37 | 58,8 | 1 | 374 |

barley. One was Maja barley (GUSTAFSSON, 1942) with six doses from 2.500—25.000 *r*, the other was a sister strain to Ymer barley (40/13 $b_7$) with nine doses from 500—25.000 *r*. In both these series the fertility was examined ear for ear and plant for plant by counting the number of ripe grains and the number of empty florets. Certainly this method is rather tedious, but it is the only fully reliable one. As Table 1 shows, when given low doses the two series agree so far as the average fertility and the number of fully fertile plants (90—100 %) are concerned. Under the highest doses Ymer $b_7$ has a lower average fertility and a lower number of fully fertile plants. On the other hand, the number of harvested plants is considerably higher in Ymer $b_7$ barley than in Maja. No doubt this partly stands in connexion with the different environmental conditions of 1940 and 1943, though different seed properties also exercise some influence.

FREISLEBEN and LEIN (l. c.) emphasize the great difference between their $X_1$ results and those submitted by GUSTAFSSON (1942). However, the differences in fertility are not very conspicuous, especially if their figures are corrected for the overlow fertility in the control line (87 % as against actually 95—98 %). The number of harvested plants, however, is considerably lower in their experiments.

A point of methodological interest is that the number of spikelets (florets) changes with the X-ray dose. The sterility is annually determined in the $X_1$ plants of two-row barley as exactly as is ever possible. These determinations give simultaneous data as to the number of ears per plant and the number of spikelets per ear. A survey is given below showing that the control material of the year 1940 contained on an average 23,98 spikelets per spike. Following a 2.500 *r* dose the number fell considerably (the difference amounts to 2,10 spikelets) in order later on to rise gradually to 27,32 and 27,07 spikelets per ear. The differences from the controls are statistically significant in the case of all doses except 5.000 *r*.

| | | Difference from controls | D/m |
|---|---|---|---|
| Control | 23,98 ± 0,29 spikelets | — | — |
| 2.500 r | 21,88 ± 0,29 » | 2,10 ± 0,41 | 5,12 |
| 5.000 *r* | 23,73 ± 0,25 » | 0,25 ± 0,38 | 0,66 |
| 10.000 *r* | 24,93 ± 0,29 » | 0,95 ± 0,41 | 2,32 |
| 15.000 *r* | 26,17 ± 0,37 » | 2,19 ± 0,47 | 4,66 |
| 20.000 *r* | 27,32 ± 0,23 » | 3,34 ± 0,37 | 9,03 |
| 25.000 *r* | 27,07 ± 0,32 » | 3,09 ± 0,43 | 7,19 |

(Maja barley X-rayed 1940; see also GUSTAFSSON, 1942 a, Table 1.)

The cause of these differences is no doubt simply as follows. FRÖIER and GUSTAFSSON (1944) have shown that the larger the X-rayed seeds are, the more will they endure irradiation; germinability and sprouting ability are better. In an ordinary sample of seeds that have not been sieved hard the smaller seeds will be the more damaged. After low doses, however, they are able to go on living, their $X_1$ plants put forth ears and set seed but, owing to their general weakness, give fewer spikelets than normally. Under high doses the X-rayed sprouts are killed early, and only the $X_1$ plants that have arisen from vigorous seeds remain alive. Their vitality enables them to develop abundant spikelets. Among other signs indicating that an enormous selection takes place is the fact that after treatment with 25.000 *r* only 8 % of all rayed seeds gave mature $X_1$ plants. The corresponding figure for the control was 75 %. An ordinary unsieved lot of seeds will show con-

siderable differences in size. As a consequence a strong selection should set in according to the X-ray susceptibility of the seeds.

In their just cited experiments FREISLEBEN and LEIN have not evaluated the fertility in the $X_1$ generation by sufficiently exact methods. Instead of the *direct sterility* ear for ear, they determined the average number of grains per plant. This is a faulty method which may lead to errors. If for some reason the $X_1$ fertility is to be analysed in order to correlate it with the type or frequency of mutation, the more roundabout method is the only correct one.

## 3. SEED PROPERTIES AND THEIR INFLUENCE ON THE X-RAY SUSCEPTIBILITY.

*Water content.* — Since STADLER's investigations (1928 b) it has been known that the mutation frequency rises considerably if the seeds are soaked in water before being irradiated. This fact has since been demonstrated by several investigators, e. g. GUSTAFSSON (1940). The increased susceptibility has been clarified by WERTZ (1940) in a careful methodological investigation. KAPLAN (1940) has studied the X-ray injuries that follow water treatment and irradiation of *Antirrhinum* pollen by determining the different germinability of the grains. (See also KNAPP and KAPLAN, 1942.)

In mutation experiments the seeds that have been soaked in water before irradiation show an inferior sprouting ability, a higher number of dead seedlings and a higher $X_1$ sterility than seeds irradiated when their water content is low. Two Svalöf experiments have made this clear. One was a comparison between series of Golden barley in which the seeds contained 10 % of $H_2O$, were soaked in distilled water for 24 hours, and treated with 0,01 % heteroauxin for the same period of time. The dose of irradiation amounted to 5.000 and 10.000 *r* (GUSTAFSSON, 1940). Series of seeds from Maja barley (1940) were also treated in the same manner. The number of harvested plants was decidedly higher in the dry-seed series than in those steeped in water, although the difference was most accentuated after 10.000 *r*. As regards the fertility, this showed a substantial fall in the water-treated series. Here, again, the differences were statistically significant. The auxin-treated and the water-treated series behaved similarly.

Small differences in water content also influence the intensity of the X-ray injuries. In the 1939 experiments with Golden barley seed samples were also rayed that contained 15 % of $H_2O$ and that had been stored at this water content for some time. The number of harvested plants did not fall notably, whereas this was the case with the mean fertility. The difference in fertility between series *B* and *A*

TABLE 2. *The fertility of the $X_1$ generation after different pre-treatment of the seeds. (Ymer $b_7$).*

| Treatment | Fertility of $X_1$ plants | | | | | | | | | | Average $X_1$ fertility | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | — 10 | — 20 | — 30 | — 40 | — 50 | — 60 | — 70 | — 80 | — 90 | — 100 % | |
| Control | — | — | — | — | — | — | — | — | — | 100 % | 96,4 ± 0,4 % | 25 |
| 80° for 72 hours | — | — | — | — | — | — | — | — | 8,0 % | 92,0 % | 94,5 ± 0,5 % | 25 |
| 80° for 144 hours | — | — | — | — | — | — | — | — | 4,0 % | 96,0 % | 94,3 ± 0,5 % | 25 |
| 80° for 216 hours | — | — | — | — | — | — | — | — | — | 100 % | 95,0 ± 0,3 % | 25 |
| 11 % $H_2O$: 10.000 *r* | — | — | 8,0 % | 10,0 % | 14,0 % | 23,0 % | 22,0 % | 16,0 % | 4,0 % | 3,0 % | 57,0 ± 1,5 % | 100 |
| $P_2O_5$: 10.000 *r* | — | 2,0 % | 6,0 % | 14,0 % | 26,0 % | 18,0 % | 14,0 % | 14,0 % | 4,0 % | 2,0 % | 53,2 ± 2,4 % | 50 |
| 80° 24 hours: 10.000 *r* | — | — | — | 1,5 % | 0,8 % | 4,6 % | 4,6 % | 17,6 % | 46,6 % | 24,4 % | 82,3 ± 1,1 % | 131 |
| 80° 72 hours: 10.000 *r* | — | — | — | — | — | 1,0 % | 4,1 % | 16,3 % | 42,9 % | 35,7 % | 85,8 ± 0,9 % | 98 |
| 80°144 hours: 10.000 *r* | — | — | — | — | 0,6 % | 1,3 % | 4,5 % | 16,0 % | 41,7 % | 35,9 % | 85,5 ± 0,8 % | 156 |

(seed material with respectively 10 and 15 % $H_2O$) amounted at 5.000 *r* to 2,96 ± 0,59 % with D/m = 5,0 and at 10.000 *r* to 6,68 ± 0,69 % with D/m = 9,7.

A desiccation of the seeds before irradiation ought to have the opposite effect to that of soaking them in water and should accordingly reduce the susceptibility. No complete experimental series have as yet been carried out in which the water content has been gradually reduced. On the other hand, an investigation was made in the spring of 1943 into the effect of X-ray irradiation combined with an intense desiccation by heat (Table 2).

A series of seed samples of Ymer $b_7$ was taken on March 17 for different pretreatments. On this occasion the water content was rather high (16,8 %). The control material was stored at room temperature, and immediately before the X-raying (March 26) the water content amounted to 11,3 %. On March 17 six boxes, each containing 300 grains in one layer, were placed in a thermostat, gradually given a temperature of 80° C. and kept there 1, 3, 6 and 9 days. Thereupon two control series and three of the heat-treated samples (1, 3 and 6 days) were given an X-ray dose of 10.000 *r*. The fertility was then determined in all $X_1$ plants that had arisen in the series subjected to combined heat and irradiation. In those control series which had been given solely 10.000 *r* 2 × 50 plants were examined for fertility, these plants being naturally taken out entirely at random.

The unrayed control series gave, as in other cases, a very high fertility (96 %). In the three series in which the grains had been merely heat-treated the fertility was somewhat reduced. Statistically, however, the differences were doubtless significant (D/m = 2,95, 3,06 and 2,88). Solely heat treatment thus reduces the fertility of the plants that arise. This in its turn is probably due to the fact that the chromosomal re-arrangements in the heat-treated seeds are considerably increased, and it agrees with earlier results obtained at Svalöf and elsewhere (see, e. g., PETO, 1937) and can be explained in a similar way as the temperature coefficient for the mutation process (TIMOFÉEFF-RESSOVSKY, 1937).

Irradiation with solely 10.000 *r* lowered the fertility from 96 % to 57 %. The great decrease of the fertility in just this experiment is probably associated with the high water content the seeds had when the samples were taken. Both the series given 10.000 *r* behaved in the same way. The average fertility in the two cases was 54 % and 59 % and the plants were distributed similarly over different fertility ranges.

X-ray treatment combined with a previous heat treatment increased the fertility very considerably, from 57 % to 82 %, 86 % and 86 % in the three heat-treated series. This is the more remarkable as heat treatment alone *reduces* fertility. The increased fertility after heat

treatment and irradiation is probably due to a loss of water in the cells, nuclei and, maybe, the chromosomes. In its turn this change brings about increased chromosome stability and fewer disturbances at the subsequent germination.

The fertility especially increased after heat treatment for three days. After treatment for 24 hours the average fertility was lower than after that lasting three and six days. The D/m for the difference between 1 and 3 days amounts to 2,53, for the difference between 1 and 6 days to 2,40.

TABLE 3. *Seed age and X-ray effect (Maja barley).*

| Treatment | Small seeds (Sieve 2,2 mm.) | | | Large seeds (Sieve 2,8 mm.) | | |
|---|---|---|---|---|---|---|
| | Per cent harvested $X_1$ plants | No. of harvested $X_1$ plants | Average $X_1$ ear fertility % | Per cent harvested $X_1$ plants | No. of harvested $X_1$ plants | Average $X_1$ ear fertility % |
| Control 1943 | 69 | 62 | 96,1 | 71 | 64 | 92,9 |
| 1942 | 67 | 60 | 96,0 | 83 | 75 | 98,5 |
| 1941 | 66 | 59 | 97,8 | 67 | 60 | 97,6 |
| 1940 | 39 | 35 | 97,7 | 33 | 30 | 98,3 |
| 1939 | 30 | 27 | 92,3 | 41 | 37 | 88,7 |
| 5.000 *r* 1943 | 46 | 139 | 76,4 | 50 | 150 | 77,4 |
| 1942 | 48 | 145 | 69,4 | 57 | 172 | 80,6 |
| 1941 | 30 | 89 | 79,2 | 50 | 149 | 83,0 |
| 1940 | 13 | 40 | 62,5 | 22 | 65 | 76,2 |
| 1939 | 10 | 29 | 55,3 | 12 | 36 | 65,3 |

SMITH, 1943, has likewise shown that heat treatment *before* irradiation increases the resistance. On the other hand, this is reduced if the heat treatment comes *after* the irradiation.

*Age.* — In 1944 an experiment was laid down to test the X-ray susceptibility in aged seed. The initial material was Maja barley seeds in two sizes (sieves 2,8 and 2,2 mm.), derived from the 1943, 1942, 1941, 1940 and 1939 crops, the samples being stored in the same way and having identical water contents. The X-ray doses amounted to 5.000 (and 10.000) *r*. Each irradiated seed-series consisted of 300 seeds, each control-series of 90 seeds. The fertility was determined within each group only on one ear per plant.

From Table 3 it is seen that the germinability of the aged control-seeds falls sharper in the small-seeded than in the large-seeded series. In the four youngest series the ear fertility is not reduced but is about 95 %.

After a dosage of 5.000 *r* the differences are very expressive. In all cases the

small-seeded series are inferior, this condition being most pronounced in material of the years 1941 and 1940. The average ear fertility is about equally high in the four youngest year-classes of each group, but distinctly reduced in the 1939 series.

Rather irregular behaviour is shown by the series at 10.000 *r*. A characteristic feature, however, is that in both the parallel series the oldest material has not yielded a single harvested plant. In the other year-classes the small seeds are considerably more susceptible than the large ones. The mean ear fertility in the series containing a sufficient material lies at 50—60 %.

*Size of seed.* — On several occasions above stress has been laid on the significance of the size of the seed for X-ray susceptibility. In the cases hitherto examined (barley, see above, also unpubl.; wheat, see FRÖIER and GUSTAFSSON, 1944) it has been shown that small though well matured and uninjured seeds are more liable to lose their vitality after even moderate doses than medium-sized seeds and these, in their turn, more easily than large seeds. The cause is probably that even at comparatively high X-ray doses numerous uninjured cells remain in the largest embryos and, on account of their livelier activity of division, replace or push aside cells that have been badly injured. It is therefore possible that the differences in X-ray susceptibility that occur in different initial materials or in different year-classes of the same strain depend at least to some extent on different seed-size. Thus, for instance, the high X-ray susceptibility in Maja barley as compared with Ymer barley is probably associated with the fact that Maja is smaller grained than the irradiated line of Ymer. The latter showed in 1943 a 1000-grain weight of 45,3 as against 42,5 for Maja.

It must not however be straightaway assumed that large-seeded varieties of a plant species would suffer less from X-ray irradiation than the small-seeded ones. This is shown by, e. g., the Svalöf spring-wheat line Kolben and the Svalöf winter wheat Sammet (Velvet) 0700. Although the latter line is much more susceptible to irradiation, its seeds have a higher 1000-grain weight (Kolben 1942: 33,3, Velvet 0700, 1942: 37,5). The two varieties have, however, a quite different descent and an entirely different physiology. Of course, other conditions than seed-size also influence the susceptibility.

Lastly, it must be emphasized that in theoretical mutation experiments and in methodological investigations it is advisable to use seeds of a certain size, this in order to reduce the variation and create as exact conditions as are at all possible.

*Autopolyploidy.* — In 1941 MÜNTZING examined the susceptibility of diploid and autotetraploid Opal B to irradiation and found that the tetraploid suffers less. As the seeds, on an average, are larger in the tetraploid than in the diploid (in 1941 the 1000-grain weights were

respectively 57,0 and 47,1), the increased resistance is probably partially due to the increased size of the seeds.

Similar results have also been obtained by LEVAN (1944) in flax. Here the strains Hercules and Concurrent, which both rank as fibre-flax, showed a reduced X-ray susceptibility in the tetraploid stage, whereas the tetraploid of Palermo, which is an oil flax, if anything behaved in the opposite way, in any case was not more resistant than the diploid. The X-ray resistance of the diploids is the highest in the large-seeded strain (the 1000-grain weight is 6,1 gm. in Palermo against 4,7 and 3,9 in Hercules and Concurrent). The highest $X_1$ resistance is shown by the tetraploid in Concurrent, next comes tetraploid Hercules, and lastly tetraploid Palermo (the 1000-grain weights are respectively 6,6, 6,4 and 7,9 gm.). Probably, therefore, both the absolute and the relative increase in seed-size have an influence on the susceptibility to X-rays.

Results similar to those in barley and flax have also been obtained in white mustard (1944 and 1945), where the tetraploid had a 1000-grain weight of 10,5 as against 6,4 gm. in the diploid. The tetraploid stands a higher quantity of X-rays. The critical dose lies between 100.000 and 145.000 *r*. In the diploid it lies at ca. 100.000 *r*.

It is a general experience that polyploid species in a genus stand a higher irradiation than the diploids, in spite of the increase in the number of chromosome disturbances. (See FRÖIER, 1946, for the Svalöf results in wheat and oats.) This is owing to the reduplication of chromosomes and genes.

The investigations hitherto conducted to ascertain the significance of the seed properties for mutation research are only a link in a systematic inquiry into the best conditions for creating valuable mutants. Other factors under investigation have reference to changes in the pH of the seeds (of great importance), protein and carbohydrate contents, influence of chemical substances (colchicine, carcinogenous substances, hormones, etc.).

## II. SPONTANEOUS AND INDUCED MUTANTS IN BARLEY.

### 1. EARLIER RESULTS.

Apart from chlorophyll mutations, which now and then appear in all rather large cultures of barley, several spontaneous mutations have been described in the literature. Dwarfs of various kinds have been observed by VESTERGAARD (1919),

MIYAZAWA (1921), HARLAN and POPE (1922) — in the last-mentioned case the dwarf resembled mut. *densinodosum*, p. 18; cf. also HONECKER (1936) — HOR (1922), HALLQVIST (1923), SCHARNAGEL (1925), and SWENSON (1940). Most of the mutants described here are recessive in relation to the maternal line. The dwarf that was isolated by MIYAZAWA was however dominant, and was fully characteristic already in the heterozygous condition. As homozygous it was but poorly viable. HONECKER (l. c.) observed a dominant or semidominant dwarf mutant. KIESSLING (1912) isolated a mutant characterized by larger foliage, longer internodes of the ears and longer awns, lighter green colour of the leaves. The chlorophyll formation was reduced. This mutant corresponds to certain *viridis* mutants which are viable when homozygous but late in ripening and low in yield.

Among morphological mutants the following are of interest. IKENO (1925) found in his experiments a dominant mutation from six-row to *intermedium* type that was constant generation after generation. TEDIN and TEDIN (1927) described a mutation changing the kernel base. In the progeny of a cross between lines having the *falsum* character (bevelled kernel base) a form of *verum* type (transversal nick) arose. This mutation took a recessive direction. MIÈGE (1927) observed a variant with smooth awns in an experiment comprising two different barley lines — »apparition brusque d'une orge à barbes lisses». It was probably due to »une mutation par perte», although the possibility of spontaneous crossing is not excluded. Both the cultivated lines were however barbed-awned. VESTERGAARD (1928) described the finding of a *zeocriton*-like variant, which had probably arisen through mutation. A transitional form to »hooded» (var. *trifurcatum*) arose in HARLAN's experiment (1931). The mutant was entirely sterile and furnished with pocket-like formations on the lemmas (similar to those in mut. *calcaroides*, p. 26). *Trifurcatum* itself is poor in yield. HARLAN considers it to have arisen as a fairly recent mutation, since in its native country it has not spread notably beyond its true area of origin (Nepal).

In a plant obtained from the cross *H. nutans* × *spontaneum* HUBER (1932) found a bud mutation that caused highly remarkable changes of the phenotype. The basal awns were thickly set with long hairs, and so were the expanded parts of the glumes. Probably this is a case of vegetative mutation confined to the epidermis, for the progeny was normal. HUBER considers that the barbs on the awns of normal barley varieties are homologous with hair formations.

Of great interest from a practical as well as a theoretical point of view is SCHIEMANN's description (1930) of a short-awned recessive mutant from the line »Bethge III». This character, which occurs spontaneously in the East-Asiatic region, was combined with a short, powerful, stiff straw and beautiful, rounded kernels. The mutant, however, had a lower yield than the maternal line. Crossed with »Bethge II» it produced a widely spread malting barley »Bethge XIII», which is considerably stiffer in straw than line II but has the same yield.

IMAI (1935) observed an interesting morphologic mutant in which the leaves of the seedling were leek-like. The root system was poorly developed, and therefore the mutated homozygotes died after a couple of months without producing ears or seeds. It was thus (sublethal or) lethal.

According to a review in Der Züchter 9, 1937, page 102, DICLUS (1936) has isolated as a mutation a f. *triaristatum*, characterized by an awn on each lemma and two awns on each palea. He also described some physiological mutants.

MARTINI and HARLAN (1942) report a series of »barley freaks», some of which were obtained after recombination; others, however, arose as spontaneous mutants. Among the latter are Grandpa, with all colour of the spike lacking (»the beard is white»), »multiflorus», with a large and varying number of flowers at each node, »accordion rachis», with long elastic zigzag curved segments.

GUSTAFSSON has observed two cases of spontaneous morphological mutations: a bud variation with half the ear formed like *distichum* (the mother-line), the other half like *tetrastichum*, and a mut. *scirpoides*. Unfortunately, the six-rowed mutated plant was lost, and therefore its heredity could not be investigated. Respecting mut. *scirpoides*, see page 31.

Waxless mutants form a transition to the physiological group. A spontaneous variant has been described by TOKHTUYEV (1935). So far back as the twenties, however, H. TEDIN (unpubl.) found a waxless mutant out of the Primus barley, which equalled the mother-line in yield.

Physiological mutants have been isolated by POPE (1935) after long selection experiments in various lines of barley. Among 35.000 plants one mutation for increased internodial length was observed (out of Deficiens), one mutation for irregularly spaced nodes (out of Hanna), and one probable mutation for changed internodial length (likewise out of Hanna).

Structural spontaneous re-arrangements in the genome have been demonstrated by SMITH (1941), who in a barley material found one plant with an inversion, which gave bridges at meiosis, one plant with a reciprocal translocation and with 65 % fertility as a consequence, and three trisomic plants with several meiotic disturbances. As early as 1932 EKSTRAND demonstrated the presence of genes for asynapsis.

The mutants that occur spontaneously are mostly lethal or sublethal, though some seem to be fully vital. This applies within the morphological as well as the physiological category. Most of the mutations are recessive in relation to the mother-lines, though at least three cases of dominants are known.

Of works on induced mutations in barley reference must naturally first be made to STADLER's (1930). Besides a large number of chlorophyll mutants he obtained seven non-seedling mutants, two of which were non-glaucous and one dormant (winter-barley like). No dominant mutation was observed.

PISMENKO (1937), cited from Der Züchter, 10, 1938, page 336, produced two mutants by irradiating ears at the time of flowering, one mutant by raying germinating seeds, and two mutants by raying dry seeds. KRAJEVOJ (1939) has given a detailed description of these five mutants: (1) *triaristatum*, which apparently is not identical with the mutant described by DICLUS under the same name but, instead, resembles my »lemma-like glumes», (2) *compactum*, similar to my *erectoides*, (3) *Pismenkovi* with a corkscrew-twisted stem, (4) *non-aristatum* with short early shed awns, sublethal, and (5) *fistulosum*, manifestly much like the mutant that is here named *scirpoides*.

KRAJEVOJ has also carried out certain minor »selection experiments» with these mutants. The best among them seems to be var. *triaristatum*. Its yield has not been tested, though its viability has, in mixed cultures with the original type. The other mutants show poor germinability and reduced viability.

By means of X-raying Abyssinian spring barley LUTKOW (1937 b) produced a mutant of winter-barley type which was recessive to the original form. Nothing

has been reported as to its cold-hardiness. He also obtained a ligule-lacking form (1937 a) and several mutants with changed ear characters (1937 c).

In 1939 NILSSON-EHLE published a preliminary report of the results he had obtained up to then with five to six different erectoid mutants. The mutations combined freely or showed linkage. Two were probably identical. One of the mutants is stated (personal communication) to yield about as much as the mother-line (Golden barley).

According to Der Züchter, 13, 1941, KRAJEVOJ (1940) produced an awnless mutant from an awned variety.

In 1941 (a and b) as well as 1942 GUSTAFSSON described a series of induced mutants in barley, some of which were rather high-yielding. Among forms of morphological interest there are some with changed spike and flower structure. Some ten erectoid mutants had been obtained up to 1941. GUSTAFSSON (1940) submits some data which were intended to indicate a non-random course of the mutation process.

Of great importance is FREISLEBEN and LEIN's find (1942) of an induced mildew-resistant mutant from the line Heines Haisa. With the aid of a specially elaborated technique for mass infection 12.000 $X_1$ offspring (comprising 240.000 seedlings) were tested for their susceptibility to *Erysiphe graminis*. Nineteen of these proved to be partially resistant and were further tested in the $X_3$ and $X_4$. A selected pedigree line entirely resisted infection by the mildew races 1, 2 and 4. In other respects the mutant was completely fertile and differed in no way morphologically from the mother-line. The yield is not yet known. These authors (1943 a, b) have also drawn up some principles for a plant-breeding by means of induced mutations.

BURNHAM (1946) found an induced recessive mutation making the chromosomes unusually long at first meiotic metaphase. Univalents were frequent. The pollen-grains aborted to 15 or 20 %.

## 2. MODEL EXPERIMENTS IN TWO-ROWED BARLEY.

Three complete series of experiments have been carried out in two-rowed barley. In the years 1932—1940 the pure line Golden barley was broken down in different directions. The same thing occurred later (1940—1943) with Maja barley, which is a homozygotized cross-product of Binder barley and Golden barley. From a sister-line ($b_7$) to the so-called Ymer barley (Victory × Maja × Opal) a series of morphological mutants was raised in 1944—1945.

Golden barley is a pure line that was placed on the market so far back as 1913. It derives from an old land barley of Gotland, was isolated at the turn of the century, and was considered suitable as initial material for the first mutation experiments. During a succession of years Golden barley was the most eminent two-rowed barley in southern Sweden, high-yielding, comparatively straw-stiff and — in its character of a pure line — completely uniform. At the end of the

twenties, however, it had to give way to the Danish strains Kenia and Opal, which combine better quality as malting barley with a higher yield and considerably increased straw strength. Even these, however, were surpassed in yield by the Maja barley, although the latter was not very suitable for malting purposes. The Ymer $b_7$ barley, finally, stands at about the same level as Maja in yield but has substantially better malting properties.

Since 1939 the experiments have had the following extent (for some of the earlier series, see GUSTAFSSON, 1940):

| | | No. of plant progenies | No. of spike progenies |
|---|---|---|---|
| 1939—1940 | Golden barley ..... | 2630 | 2630 |
| 1940—1943 | Maja barley ..... | 2256 | 9681 |
| 1943—1945 | Ymer barley ($b_7$) .. | 2010 | 3345 |
| 1939—1945 | Controls | 626 | 887 |

The X-ray doses ranged from 500 to 25.000 *r*. Dry seed and seed pretreated in different ways were X-rayed.

The mutants produced in the three varieties of barley may be phenotypically divided into three groups: (1) chlorophyll mutants, (2) sterility and lethality mutants, and (3) vital mutants.

### A. CHLOROPHYLL MUTANTS.

This group of mutants is especially suitable for methodological investigations in diploid plants. Chlorophyll mutations readily arise and can be discovered at seedling stage. The majority are lethal, live as long as the store of nourishment lasts, and then die. They should therefore really be assigned to Group 2 but are conveniently separated from this. A few are subvital, i. e. are able to assimilate, form ears and even ripe kernels, although the ripening is often greatly retarded. In one case there even arose a viable chlorophyll mutant that under certain external conditions seemed to be superior to the original form in yield. In flax LEVAN (1944) has produced a *viridis* mutant that is fully vital also under normal external conditions (p. 85). The mutants can be placed according to their appearance and chlorophyll formation in different subgroups (GUSTAFSSON, 1940; see also FREISLEBEN and LEIN, 1943, as well as FRÖIER, 1946).

The plastids are as a rule normally or subnormally developed in *viridis* mutants of barley, whereas they are small and undeveloped in

*albina* types. Plastids of the *xantha* types occupy an intermediate position. In the *alboviridis* mutants they often develop differently at the apex and base of the leaves (GUSTAFSSON, 1942 b). For oats FRÖIER (l. c.) has observed a somewhat deviating behaviour. The commonest of the mutant types in barley are *albina*, *virescens*, *chlorina*, *virido-albina*. Sporadically *alboxantha*, *xanthalba*, and *tigrina* arise.

### B. STERILITY MUTANTS.

These mutants form two subgroups.

The *first* comprises such mutants that already have reduced viability in the vegetative stage and consequently poor seed-setting, but also mutants that as a result of altered spike or flower structure are wholly or partially sterile.

Dwarfs with an enfeebled development and partial fertility are not infrequently formed. In the cases examined they remain constant year after year, the ears burst their envelopes incompletely and the seed production is reduced. Narrow-leaved variants with dwarf growth are not uncommon.

In a peculiar mutant that arose from Ymer $b_7$ in 1945 most of the spikelets were sterile and seed was only developed in the very highest flowers. A similar mutant was described by VESTERGAARD (1928).

One characteristic mutant has repeatedly ramified ears (GUSTAFSSON, 1941, Fig. 7). It is a compact dwarf with numerous nodes and short internodes. Its pollen is normal but in spite of this the plants are entirely sterile. This type of mutant has arisen in Maja as well as Ymer $b_7$ and seems to be identical with a form described by HARLAN and POPE (1922). It has been given the name of *densinodosum*. SMITH (1939) has found a similar mutant in *Triticum monococcum* (*mi mi*, his Fig. 11), although it was much higher and, in addition, partially fertile.

To this group there also belongs a mutant that has been described by GUSTAFSSON and ÅBERG (1940). The spikelets of the middle-row contained two flowers within each lemma. The long-awned primary lemma looked normal. The two secondary lemmas possessed awns about three-fourths the length of the ordinary ones. Similarly the paleae had awns about 2 cm. in length. Frequently the secondary lemmas grew together. Seeds were never formed, although the pollen looked normal. This mutant type has been found several times. Owing to its complete sterility it has not been specially studied.

SMITH (l. c.) has found several cases of diplontic sterility in *Triticum monococcum*, among others anther-less mutants, where the anthers ceased development before or shortly after meiosis was completed.

The *second* group of sterility mutants do not produce seed or do so incompletely as a consequence of disturbed meiosis, caused by recessive factors. SMITH observed many such cases in the progeny of X-rayed *T. monococcum*. Sometimes the chromosomes remained unpaired (partial or complete asyndesis), sometimes they were fragmented at first division, in other cases diploid microspores arose, diakinesis was retarded, etc. In some cases pollen was formed but aborted owing to the mature grains lacking starch. The recessives may be vegetatively normal, but also very weak.

### C. VITAL MUTANTS.

This term includes here such variants as in a vegetative respect do not show any lethal traits and, in addition, are entirely fertile. Vital mutants in barley may grade below, level with or lie above the original line in yield. In the last case they may be called progressive. Even a variant that is inferior to the original line in the latter's proper area of cultivation may occasionally become distinctly superior to it in another climate or another environment, in which case it must also be called progressive.

For the sake of simplicity two subgroups may be distinguished within the group of vital mutants (GUSTAFSSON, 1941 a): (a) *Morphological mutants*: morphological characters are altered in addition to changes in physiological properties. As a rule they segregate sharply in intercrossings or in crossings with the original line; they are qualitative. (b) *Physiological mutants* which, when cultivated alongside the original, can be distinguished from it, but only in properties relating to straw-height, straw-stiffness, earliness, tillering capacity, etc. In the crossings hitherto studied there is no sharp segregation. The $F_2$-segregates cannot be divided into two (or three) distinct classes, the phenotypical variation covering the genotypical. Most of BAUR's or TINE TAMME's »Kleinmutationen» belong here.

There is no plain difference between the subgroups. Some winter barley-like mutants, which segregate sharply and are easily distinguished both from heterozygotes and from normal homozygotes by their extreme lateness, form a transition between the two subgroups. The extreme lateness (i. e. the winter-barley character) is rather a phy-

siological property. In the cases studied, however, it was combined with obvious changes in morphology.

### a. Morphological mutants.

The so-called erectoid mutants are the commonest within the morphological group. In Golden barley ten such mutants have been induced (*erectoides* 1, 2, 4—11). Of these, *erectoides* 8 and 10 have

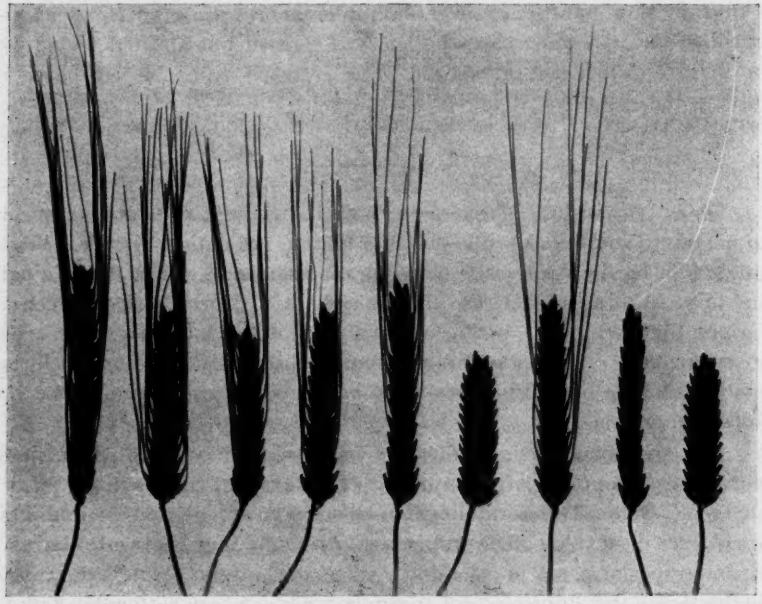


Fig. 1. To the extreme left Golden barley (the mother strain), then the mutants *erectoides* 1, 2, 4, 5, 6, 7, 9, 11.

been lost. (*Erectoides* 3, mentioned in earlier papers, is probably an intermixture.) In Maja barley five mutants of erectoid type have been obtained (12—16) and in Ymer barley, finally, nine (*erectoides* 17—19, 21—24, 27, 28). (The three mutants »*erectoides* 20, 25 and 26», which at their first appearance were placed in this group, fall outside it in spite of having certain characters in common with it.) Something similar may also be said of *erectoides* 16 (Maja). An interesting fact is that NILSSON-EHLE (1939) observed six mutants of erectoid type, arisen out of Golden barley, but no other morphologically deviating

forms. In my own experiments, irrespective of the erectoid types, about 20 vital morphological mutants representing some ten different types have been obtained. Thus, the erectoid type arises oftener, and, as will be shown later, also more readily than most of the other morphological mutants.

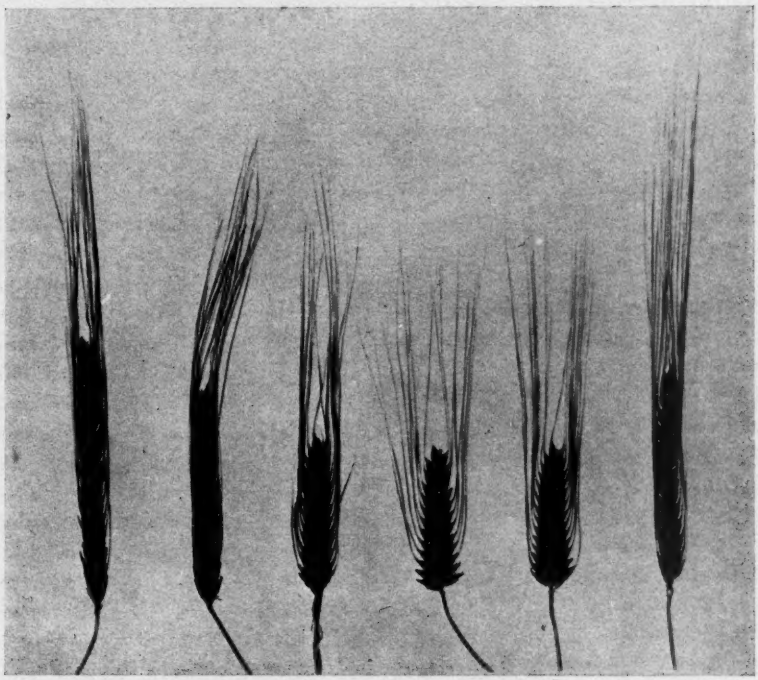


Fig. 2. From left to right: Maja barley (the mother strain), *erectoides* 12, 13, 14, 15, 16.

All erectoid mutants (Figs. 1—3) are characterized by comparatively short, compact ears, often with projecting kernels and awns. A great variety of gradations occur, from subnormal to very short, compressed ears, the last-mentioned resembling those in *zeocriton* barley. The ripe kernels in the two lowest florets often stand straight out from the main axis, thus giving the ears a characteristic appearance, tapering from below upwards. This compact ear type is often accompanied by a short straw. The ears are shortened more than the straws.

In the Golden-barley mutants the relative ear length is 81, in the Maja mutants 77 (both the original lines put to 100). The straw-length amounts to 97 and 93 respectively as against 100 in the controls. Some mutants break this correlation. The most striking is *erectoides* 14,

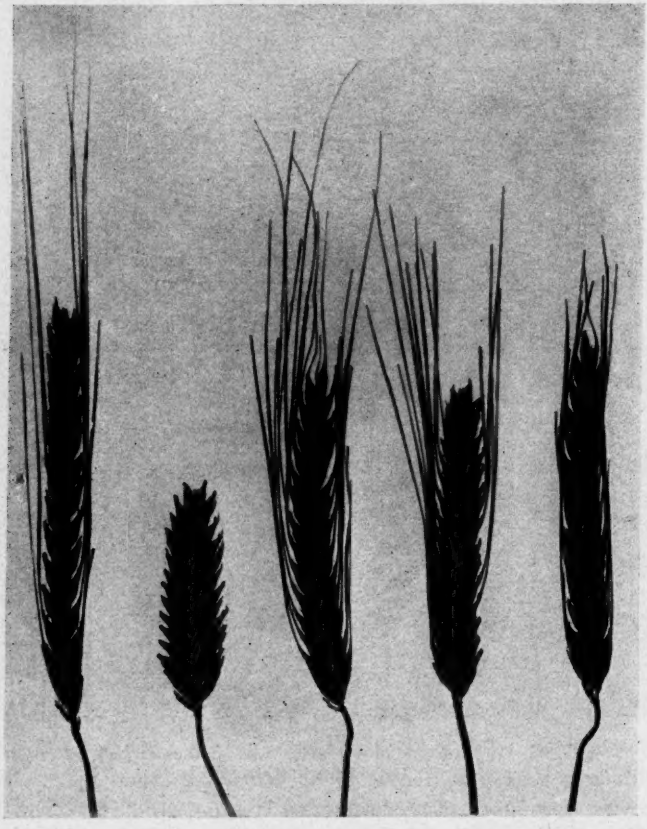


Fig. 3. From left to right: Ymer barley 40/13 $b_7$ (the mother strain), *erectoides* 17, 18, 19, and a short-awned mutant.

which in spite of its extremely compact ears possesses a substantially longer straw than the Maja barley (diff.$_{straw}$ $= 7{,}0 \pm 1{,}6$ cm., D/m$=4{,}5$; diff.$_{ear}$ $= -2{,}5 \pm 0{,}2$ cm., D/m $= 10{,}3$). Thus, the two systems can be changed by way of mutation in opposite directions. *Erectoides* 6 and 11 may also to some extent be ranked as correlation breakers; they

have the most compact ears in the Golden-barley group but the straws are only slightly shorter than in the original line.

The erectoid mutants that have arisen from Maja barley show a considerably stronger variation than those arising from Golden barley. The relative figures for the ear-lengths vary in the Maja mutants between 57 and 97, in the Golden-barley mutants between 70 and 91. The relative figures for straw-length lie in Maja between 70 and 111, in Golden barley between 87 and 101.

The erectoid types possess on an average a lower number of kernels (and with this spikelets per ear) than the original types, but the difference is not considerable. The same applies to the 1000-grain weights. In both groups the mutants fluctuate round their mother-line. Only *erectoides* 16 possesses an appreciably higher 1000-grain weight. The, broadly viewed, constant spikelet numbers and 1000-grain weights, coincident with much shortened ears, obviously explain the projecting appearance of kernels and awns.

In the examined erectoid mutants the straw-strength is always increased. The Golden barley types further differ from the mother-line by upright ears, in the original type they bend downwards. Maja barley and its mutants have upright ears. Even *erectoides* 14, which has acquired a taller straw, shows a distinctly increased straw-strength. The increase is greater, however, in *erectoides* 13, 15 and 16, which are so extremely straw-stiff that under the Svalöf conditions they surpass most other barley lines.

Certain erectoid mutants also deviate from the original lines in earliness. The figures from the yield trials of 1944 may be submitted here. These trials included *erectoides* 1, 7 (from Golden barley), 12, 13, 14, 15, 16 (from Maja barley). *Erectoides* 1, 12 and 13 were this year (as well as other years) about equally early in earing and maturing as the mother-line. *Erectoides* 7 and 15 were distinctly later, two and two to three days respectively. *Erectoides* 14 was a little earlier (two days). *Erectoides* 16 is very remarkable, it ripens extremely quickly. It shoots almost a week before the Maja barley. In the extensive yield trials of 1944 and 1945 it matured about seven days earlier. Whereas in most of the spring barley lines the seedlings remain for a time in a vegetative strengthening stage, in *erectoides* 16 they shoot up direct.

The pronounced pleiotropy shown by many erectoid mutants does not however cease with this. The various barley lines can be divided into an $\alpha$ or $\beta$ type, or, as the case may be, a $\gamma$ or $\delta$ type, depending on whether they are devoid of teeth on the lateral nerves of the lemmas

or in possession of such (ATTERBERG's system). Golden barley is a $\beta$ type with 5—7 teeth on each nerve, whereas Maja barley is most closely an $\alpha$ type, although solitary teeth may be formed. *Erectoides* 1 resembles Golden barley in this respect, while *erectoides* 4 and 5 clearly deviate. They possess on an average about three teeth per nerve. *Erectoides* 9, however, has if anything a higher number, about eight teeth per nerve. Most of the Maja mutants agree with the mother-line, but *erectoides* 14 has more teeth ($\beta$ type).

The shedding of the awns varies greatly in different mutants. As Fig. 1 shows, *erectoides* 6, 9 and 11 are very apt to shed their awns. Among the Ymer mutants this applies in a specially high degree to *erectoides* 17 (Fig. 3). Attention has been directed on several occasions to the peculiar mutant *erectoides* 16. In this, the erectoid character does not always appear so distinctly; the length of its ear to that of the mother-line bears the ratio 97 : 100, but the lowest grains often project so much that the name can be defended. It deviates from Maja slightly in the dentation of the lemmas but considerably in 1000-grain weight, earliness and straw-strength as well as in regard to its awns, which long before maturing are deeply stained with anthocyanin and are rust-brown in colour.

The examined erectoid types are recessive to the mother-line. In some cases the factors are linked. Free combination gives double recessives of the *zeocriton* type. In several cases, independent in origin, the original line has given identical erectoid mutations.

Among the other morphological mutants mention may here first of all be made of two in which both the glumes at each spikelet were converted into lemma-like formations, each with its awns (»lemma-like glumes» 1 and 2). From each spikelet, therefore, there issue three long awns. The mutant has been described by GUSTAFSSON and ÅBERG (1940). It was observed earlier in India and the U. S. A., having presumably also arisen there by recent mutation, and was described by PISMENKO (1937) and KRAJEVOJ (1939) as induced by X-rays. The two Svalöf mutants have been observed in Golden barley.

The glumes have changed in exactly the same way in both cases. The change is probably conditioned by one and the same gene (or by multiple alleles), for the $F_1$ is identical with the parents and no segregation occurs in the $F_2$. One is undoubtedly recessive to the original line and the 3 : 1 segregation is regular. Still, the two mutants are by no means identical. They differ clearly in respect of straw-height and ear-length. They were cultivated in the year 1944 next to each other

and with the original line in the vicinity. One was shorter (diff. = = — 17,8 ± 1,6 cm.) and the other taller than Golden barley (diff. = = 4,2 ± 1,3 cm.). Under the conditions of 1944 the two mutants differed in height by not less than 22 cm. In ear-length the mother-line lies midway between the two mutants. Mutant 1 has a shorter ear (diff. = — 0,44 ± 0,27 cm.), mutant 2 has a longer ear (diff. = = 0,48 ± 0,28 cm.). The mutants are thus not definitely separated from the mother-line, though distinctly from each other (diff. = 0,92 ± 0,26 cm., D/m = 3,5). Mut. 1 is richer in anthocyanin than mut. 2; this stands out most clearly in the awns. In addition, it has coloured nerves on the lemmas. Mut. 2 in its turn deviates as regards the teeth on the lemmas; they are certainly not more numerous but are decidedly stronger. Both the mutants have a higher 1000-grain weight than Golden barley.

Of great morphological interest is the circumstance that the lemmas in the lateral rows in both the mutants may be pointed and provided with short awns. In mut. 1 rudimentary lateral flowers with awned lemmas rather seldom occur, in mut. 2 this is commoner and the awns are longer. The very pleiotropic character of the two mutations thus strikes the eye immediately. Especially remarkable is the fact that the straw- and ear-length increases in one mutant but diminishes in the other.

Mut. *intermedium* once arose from Ymer barley. HOFFMANN (see ISENBECK and HOFFMANN, 1942) divided the cultivated barley (*Hordeum sativum*) into four main series: *H. polystichum* (= *H. vulgare*), *H. intermedium*, *H. distichum* and *H. deficiens*, according to the number of fertile spikelets on each node [1]. In *H. intermedium* one, two or three spikelets set mature seed alternatively. The middle row is always fertile and better developed than the side rows. The mutant that has arisen at Svalöf corresponds to the description. According to VAVILOV and ORLOV, similar forms occur spontaneously in Abyssinia and Arabia. Such have also arisen by spontaneous mutation (IKENO). *H. polystichum*, *distichum* and *deficiens* form a multiple-allele series. The factors that control the *intermedium* character lie outside this series. None the less, *polystichum*- and *distichum*-forms, crossed with one another, can give *intermedium*-like segregates in the $F_2$ and $F_3$. According to ISENBECK and HOFFMANN (l. c., p. 148), these arise when

[1] The system recently introduced by ÅBERG and WIEBE (1946) could not be considered here.

special fertility-factors are combined with the factor for two-rowedness. IKENO's *intermedium* mutant dominated over the six-row type. The mutant described here has not yet been crossed with the original form or with other two- and six-rowed barleys.

Six-rowed (i. e. *polystichum*-) forms have arisen a couple of times, once with certainty out of Golden barley, once out of Ymer barley. Both were first observed in heterozygous form; the side flowers were accordingly more developed than is the case in ordinary two-rowed barley and their lemmas were provided with distinct awns. The former mutant was fertile and gave rise to six-row homozygotes of normal appearance (*tetrastichum* type) but with weak tillering capacity. The other case was associated with complete sterility, and therefore no progeny could be obtained.

A very peculiar mutant, which excellently illustrates how a mutant can alter the morphology of the spikelet, arose out of Ymer barley in 1944 and has been tentatively named mut. *calcaroides* (Fig. 4).

In this mutant the upper part of the lemma arches downwards and forms a sack-like, often even spur-like, recess. The awn is attached to the exterior part of the spur. On account of the shape of the sack the inside of the spikelet comes to lie exposed. In spite of this, self-fertilization seems to predominate, for the progenies from the homozygous calcaroid plants, discovered in the summer of 1944 and surrounded on all sides by normal Ymer $b_7$ plants, were homozygous throughout in the $X_s$ as well. The lowest parts of sack and awn are very hairy, sometimes almost hirsute. The barbs on the awns are thinner than in Ymer barley and resemble hairs. The lemmas of the lateral spikelets are larger than in the original type. The kernel base does not slope as is normal in nutans barley but is more sharply inclined (spurium-like). There is considerable variation in the appearance of the sack; the latter may be displaced to the side, upwards or downwards, the recess may be of different depth, sometimes almost vanishing, the awn is differently attached, etc. The pleiotropism thus appears very clearly in this case.

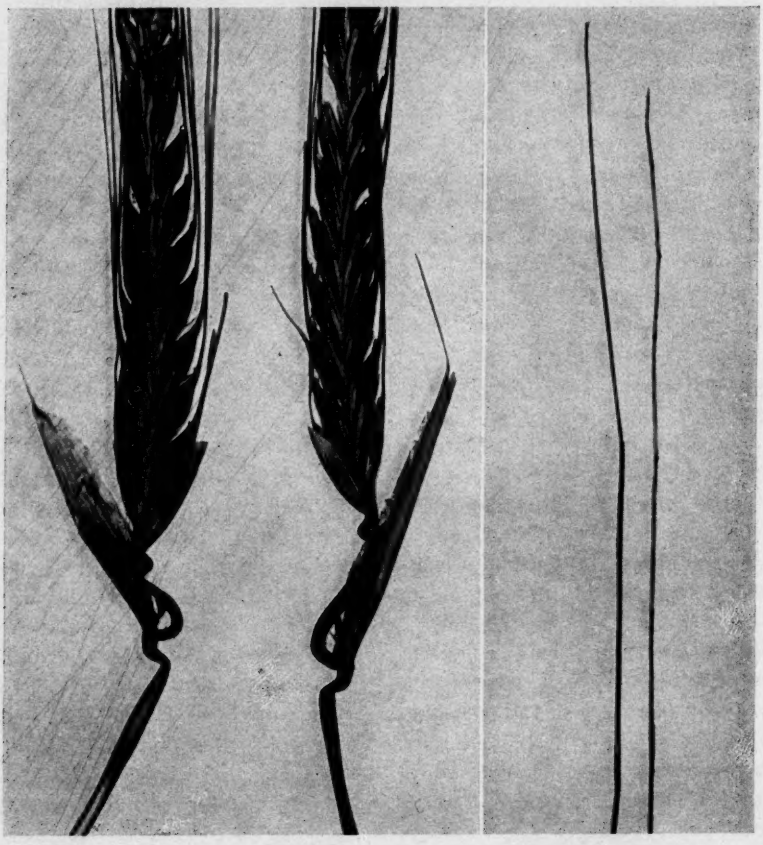
In the progenies that were raised in 1945 from three calcaroid plants the leaves withered away before ripening. Whether the calcaroid characters pleiotropically control this chlorophyll-defect or whether two different mutations have come together by chance, cannot be settled as yet. Calcaroid homozygotes are vegetatively rather feeble in segregating progenies.

This mutant shows striking similarities to a sterile variant that

Fig. 4. Mut. *calcaroides*. Note the spur-like upper parts of the lemmas. This and the mutant in Fig. 5 have arisen from Ymer barley $b_7$.

arose in HARLAN's experiments (1931) and that was considered to form a transition to the so-called *trifurcatum*.

In Ymer barley there likewise arose in 1944 a peculiar mutant,



*a* *b*

Fig. 5. Mut. *bracteatum*. — *a*. Note the large extra-bracts below the inflorescence, the gradually narrowing bracts in the inflorescence, and the curved culms. — *b*. To the left: the normal appearance of a barley culm (the mother strain), to the right: note the numerous short internodes of the mutant.

tentatively named mut. *bracteatum* (Fig. 5). From extra nodes under the ear there issue bract-like leaves that form a tunic in which the ear is enclosed. In the axils of these bracts are rudimentary, sterile flowers.

From the node under the spikelets there spring successively ever-shorter bracts, and these gradually become scale-like. They are hairy. At the middle of the ear they disappear. The uppermost portion of the culm is curved. In the autumn of 1945 homozygous mutated plants exhibited a peculiar behaviour, folding at the curved section that occurs just beneath the ear and causing this to hang slackly down, though without breaking the culm. The number of nodes on the culms is substantially increased. The two uppermost internodes in normal barley are represented in this mutant by four or five (Fig. 5 *b*), which become successively shorter.

The homozygous plants are vegetatively robust and very waxy.

From Ymer $b_7$ there arose in 1944 another peculiar mutant (Fig. 3) that is striking *on account of the short awns* on the lemmas. The mutation is pleiotropic. The culm is shorter, besides being more waxy and much stiffer than in the original line. The auricles are highly anthocyanin coloured. The ear has a weak erectoid character.

From both Maja barley and Ymer barley some mutants have been produced that are characterized *by a peculiarly glaring green colour* and are at the same time entirely or partially waxless. Analysis of the $X_3$ shows that they are recessive to the normal type and that the heterozygotes segregate simply. Whether all the four mutants are controlled by one or several factors, has not yet been determined. The two Maja mutants are mutually alike. The mother-line, as mentioned earlier, is of the so-called $\alpha(-\beta)$-type, one of the bright-green mutants, on the other hand, of $\beta$-type. Both have a shorter straw (59,8 and 59,4 cm. as against 65,8 cm. for Maja). The ear-length is practically the same, however, and so is the number of spikelets per ear. The mutant that has given the lowest yield has a substantially lower 1000-grain weight. A spontaneous mutant that is devoid of wax but has not the same peculiar bright-green colour arose earlier in Svalöf (out of Primus barley, H. TEDIN).

In an earlier paper (GUSTAFSSON, 1941 b) reference was made to a mutant out of Golden barley *with half-naked kernels*. At ripening and still more during threshing the lemmas drop from the caryopses. The nakedness is incomplete. This mutant, which was found to have a poor yield, is easily attacked by smut. Certainly it is stiffer in the straw than the original line, but it has considerably smaller and less developed kernels. These possess a high protein content and exhibit a faster germination ripeness than the mother-line.

Two mutants that have arisen out of Golden barley have been

denoted as *winter-barley like* (GUSTAFSSON, 1941). They shoot about one month after the original line and as seedlings remain longer in the vegetative stage. Sown in the autumn, they deviate considerably from

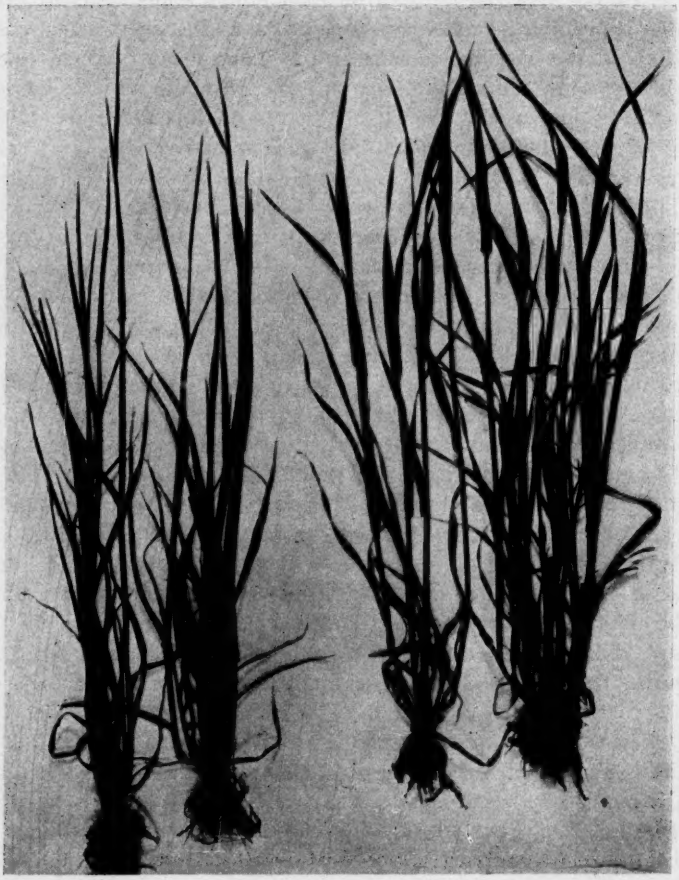


Fig. 6. To the left: two plants of the spontaneous mutant *scirpoides* showing involute leaves. To the right: two plants of Golden barley (the mother strain).

ordinary winter-barley lines in that the seedlings quickly grow out (contrary to the case in the spring) and die early on account of poor cold-hardiness. They are thus characterized rather by extreme lateness than by real winter-barley type (altered photoperiodism?). In one of

them (winter-barley 1) the seedlings are yellowish-green at first and for this reason presumably possess lower assimilation intensity. The other mutant (winter-barley 2) has an almost reed-like appearance on earing. It is tall and robust with very broad leaves. The heights in 1944 of Golden barley, winter-barley 1, winter-barley 2 were respectively $78{,}7 \pm 0{,}9$, $63{,}5 \pm 0{,}9$ and $87{,}3 \pm 1{,}7$ cm., and in certain years the differences were still more pronounced. Nevertheless, the ears are considerably shorter in winter-barley 2. The two mutants are more liable to shed their awns than the mother-line and have a lower 1000-grain weight than the latter. Besides these characters the two mutants also deviate in respect of number of teeth on the lemmas, which is considerably lower than in Golden barley itself. Winter-barley 2 is further characterized by the fact that certain lateral flowers have pointed lemmas, winter-barley 1 by coloured nerves on the lemmas.

Finally, mention must be made of a morphological mutation which, although not having arisen in the progeny from X-rayed seeds, appeared in the $F_2$ of the cross *erectoides* 7 $\times$ *erectoides* 6, both X-ray mutants. It has been named mut. *scirpoides* (Fig. 6). Before the earing the erect leaves, which enclose the spike, give a peculiar appearance to the mutant. The leaves are involute, subulate and yellow at the apex. Possibly the mutation has arisen as a result of chromosomal rearrangements which were induced at the origin of the parents and which have caused pairing disturbances in the double heterozygote (?). Although both the parent lines have been cultivated on a large scale, neither of them has given mutations in its progeny. Three of seven $F_2$ progenies segregated in both the erectoid and the scirpoid character. The reciprocal combination, which consisted of ten $F_1$ plants, did not give any scirpoid types. The scirpoid phenotype is controlled by a Mendelian factor which seems to segregate independently of the two erectoid factors. It has admitted of being transmitted without difficulty to normal nutans barley (*AABB*), the two single recessives (*AAbb* and *aaBB*) of erectoid type as well as to the double recessive (*aabb*) of the *zeocriton* type.

### b. Physiological mutants.

The physiological mutants, like the morphological, affect all organ systems, although they chiefly have a quantitative character and do not deviate from the maternal line so much that progenies from crosses can be assorted into distinct classes. None the less, in this group there

are also mutants which, cultivated side by side with the original line, differ considerably.

Outwardly the most deviating is perhaps a mutant out of Maja barley (44/98) with a greatly shortened straw. At measurements in 1944 the straw was reduced by 40 %, from 64,2 to 38,8 cm. (D/m = 30,2). The ear has likewise become shorter, though not in the same proportion, the shortening here being from 7,9 to 6,7 cm. Still, the difference is statistically significant (D/m = 7,0). The shortening is due to there

Fig. 7. Winter barley-like mutant (No. 2). In the back-ground Golden barley. Photographed on July 9th, 1941.

being fewer spikelets, not to shorter internodes as in the erectoid mutants. A considerably lower 1000-grain weight falls to the mutant than to the mother-line (35,7 against 43,2). There is good tillering capacity. The mutant belongs to the *a* type like the mother-line but, like this, has one or two solitary teeth on the lemmas.

The least changed in appearance of the isolated mutants, (44/23), deviates in leaf-breadth and 1000-grain weight as well as — and above all — in malting qualities (p. 73). In other respects it seems to be identical with the mother-line (Golden barley). Its morphological characters are the same, and so are its straw-height, straw-strength, ear-length and earliness. In yield it shows about the same level as

Golden barley. The leaf-breadth [1] in the mutant was 11,7 mm. in 1944 as against 10,1 in Golden barley, the difference being statistically significant (D/m = 7,3). Somewhat shorter leaves mark the mutant (diff. = 1,77 ± 0,61 cm.). In the yield trials of 1943—1945 the average 1000-grain weight amounted to 44,4 against 40,4 for Golden barley. This mutant arose in the same pedigree progeny as the extremely broad-leafed winter-barley like variant (No. 2).

In these two cases the mutants differ distinctly from the maternal

Fig. 8. To the right: an extremely straw-stiff and bushy mutant (44/3), to the left: a straw-weak mutant (44/4). Yield experiments (1942).

line only in one external character. Other mutants, however, deviated in many ways. Especially interesting is a type that was obtained in 1939 from Golden barley (44/3). It differs much from all barley lines growing at present at Svalöf. The seedlings early become very bushy and at the same time extremely waxy. The ear does not slope as in Golden barley but is erect, and the culm is considerably shorter. A most remarkable feature is the extreme straw-strength, in Svalöf evaluated in 1944 and 1945 at 9,3 as against 6,2 for Golden barley. This relation has held year after year, even in other areas of cultivation. In 1945

[1] In this as well as other cases the leaf-breadth was measured three weeks after earing on the second leaf of the main shoot, counting from the top.

such large quantities of rain fell in the month of July over the area in which the branch station of Västernorrland is situated that even the otherwise straw-stiffest strains were felled to the ground. Only this mutant kept upright. The ripening time is about the same in the mutant as in Golden barley, if anything shortened by one or two days. Both mutant and mother-line belong to the $\beta$ type. The extreme straw-strength is counteracted by two bad properties, a low 1000-grain weight and an inferior malting quality. Probably the low 1000-grain weight is due to the fact that the awns drop off to a large extent before ripening, just at a time when they are still able to assimilate. In yield the mutant lies at practically the same level as the maternal line. This interesting mutant reveals one of the disadvantages of the X-ray method: while good »progressive» properties can certainly be induced, changes often arise that reduce the value of the mutant.

Increased vegetative development, specially *higher straw and retarded ripening*, are often combined. Only seldom, however, are the changes so extreme as in the winter-barley like type 2 (p. 31). The culm may be some centimetres higher than in the mother-line, earing and ripening may occur a couple of days later.

One of the oldest mutants in cultivation, (44/2), differs from Golden barley chiefly by higher straw (in certain years up to ten centimetres) and two to five days later maturation. In spite of the higher straw the straw-strength is about as good as that in the mother-line. The 1000-grain weight is generally somewhat higher than in Golden barley, amounting for the five years 1940—1944 to 44,9 as against 43,9. The yield exhibits a tendency to rise during dry summers and to fall during rainy ones (p. 64). Thus, the mutant appears to be more resistant to drought than Golden barley.

Mutants with increased straw-height and lateness have been encountered several times in both Golden and Maja barleys. These two characters are often combined with *increased leaf-breadth, lighter green colour, reduced waxiness.*

The *straw-strength* not so infrequently changes, in a positive as well as a negative direction. As was earlier mentioned, most of the erectoid mutants possess increased straw-strength. A very straw-stiff physiological mutant that arose out of Golden barley has been previously mentioned (the waxy mutant 44/3). Such variants have also been produced from Maja barley. A Maja mutant (44/18), which has now been tested in yield trials for two years and is equal to the maternal line in yield, differs by high straw-strength (8,2 against 7,5), somewhat

higher straw (diff. = 5,5 ± 1,2 cm., D/m = 4,5), greater waxiness, somewhat higher 1000-grain weight and, above all, increased earliness in earing as well as ripening (2—4 days). In another mutant (44/30) the straw-strength increased still more strikingly (8,9 against 7,3). The straw-height, 1000-grain weight and earliness were in this case exactly the same. On the other hand, the colour of the ripe kernels was different, more yellowish, the kernels as well as in fact the whole plant not being so rich in anthocyanin.

On several occasions mention has been made of mutants that combine an increased or decreased 1000-grain weight with other changes. Especially remarkable among these *large-kernelled mutants* is a type out of Golden barley (44/7) with an average 1000-grain weight for the years 1941—1945 of 46,4 as against 41,2 for the original line, i. e. an increase of 13 %. This mutant also differs in other respects. The colour of the kernel on ripening is more yellowish-brown, although in other ways the mutant is quite as rich in anthocyanin as the mother. It also has somewhat broader leaves. In straw-height, straw-strength, earliness, etc. it deviates only slightly and has the Golden-barley habit in other respects. The malting properties are improved. Similar variants have also been obtained from Maja barley. One of these (44/91) possessed in the spring of 1945 a 1000-grain weight of 52,0 gm. as against the 46,1 gm. of the Maja control. As in the preceding case, the kernel colour had changed to a distinctly yellowish-brown tone. The straw was substantially higher (diff. = 9,8 ± 1,3 cm., D/m = 7,7) and the ear was considerably longer (diff. = 1,5 ± 0,2 cm., D/m = 7,2) than in the mother. Simultaneously, however, the straw-strength had fallen considerably. The kernels belong to the *a* type.

Several mutants with *increased earliness* have been dealt with in the previous text. The most interesting from a practical point of view is *erectoides* 16 (p. 23), which ripens at Svalöf quicker than other Scandinavian barley lines and is as early as the early Australian and Egyptian types included in the assortment. (It differs entirely from these, morphologically among other things by the anthocyanin-coloured auricles.) Among the physiological mutants certain variants from Golden barley are earlier than the maternal line, although only to the extent of one or two days. One of them, the waxy mutant (44/3), has already been discussed. Reference has also been made earlier to one of the early physiological mutants (44/18) out of Maja barley.

The results show that changes are more liable to occur towards increased lateness than in the opposite direction.

In GUSTAFSSON (1941 b) some results relative to the process of *germination ripeness* have been discussed. Changes in this character were attended by alterations in various morphological and physiological characteristics. No selection for solely accelerated or delayed germination ripeness has been made.

Respecting properties such as *protein and starch content* as well as *malting properties*, see page 69.

## III. THE FERTILITY OF THE MUTANTS. SYNTHETIC STERILITY.

The vitality of a mutant is determined to an essential extent by its seed production. This in its turn is bound up with the ear fertility, number of spikelets on an ear, and the tillering capacity. The germinability is also of importance.

The direct *fertility* has been determined for eight morphological mutants from Golden barley — all belonging to the *erectoides* type. This was done by counting the number of unfertilized florets and number of developed kernels on the main straw of 25—75 plants taken at random. The fertility of such commercial varieties as Golden, Maja and Ymer barleys usually amounts to 96—98 %. On this occasion Golden barley showed a fertility of 98,0 %. The lowest value for any mutant amounted to 96,1 % (*erectoides* 2) and the highest to 98,7 % (*erectoides* 7). The D/m varied between 0,02 and 1,87. Thus, none of these mutants have a notably reduced fertility. Where sterility occurs, it does not amount to more than a few per cent. The same applies to the great majority of pure-bred mutants that have been tested in yield trials [1]. As a matter of fact, during the last few years only such mutants have been taken out as, judging by ocular inspection, are fertile. Hence a conscious selection works here in the service of the practical work.

*The number of kernels* produced by an ear is determined not only by the sterility but also by the number of spikelets (florets). If no sterility occurs — as in the above-mentioned erectoid mutants — the number of kernels is determined by the number of spikelets that have

[1] Five morphological mutants out of Ymer $b_7$ have been examined in the $X_4$ (1945) in regard to their fertility. The maternal line showed an average fertility of 96,0 %. *Erectoides* 19, »bright-green» 4, and »short-awned *erectoides*» gave respectively 96,6, 98,1 and 94,4 %. Two other mutants (*erectoides* 17 and 18) contained, however, a number of plants with reduced fertility, the average figures being 79,9 and 85,3 %. The material was as yet not entirely homozygotized for structural re-arrangements.

been formed. Even the commercial strains exhibit differences in this respect. For instance, on analysis in the summer of 1945 Maja barley gave an average of 26,4 spikelets per ear, Golden barley an average of 24,2. The difference is $2{,}3 \pm 0{,}7$; $D/m = 3{,}3$. In one trial in 1944 the number of ripe kernels per ear in the same strains amounted to 26,8 and 24,2 (difference $= 2{,}6 \pm 0{,}4$; $D/m = 6{,}8$). The higher yield of the Maja barley compared with Golden barley largely depends on a greater number of spikelets.

In the examined mutants a clear group difference is observed according to their descent. Ten morphological mutants out of Golden barley showed an average of 23,9 kernels per ear against 24,2 for the maternal line, seven morphological and fifteen physiological mutants from Maja barley gave 26,1 kernels per ear against 26,8 for Maja itself. The kernel numbers of the two mother-lines show a ratio of 100 : 111, those of the two mutant groups, 100 : 109 — hence a strict parallelism[1].

The individual mutants differ by the following D/m values from the mother strains:

| | Fewer kernels per ear | | | | More kernels per ear | | | | |
|---|---|---|---|---|---|---|---|---|---|
| D/m | — 3 | — 2 | — 1 | — 0 | — 1 | — 2 | — 3 | — | |
| Number of cases | *8* | 1 | 6 | 4 | 4 | 2 | 4 | *3* | = 32 |

Thus, in every *fourth* vital mutant examined the number of ripe kernels per ear has been so reduced that it definitely differs from that of the maternal line ($D/m = 3$). In every *eleventh* mutant it has increased in a corresponding degree. Negative as well as positive mutants may thus arise. In all cases, i. e. even in those with high D/m values, there has been only a slight decrease (or increase) in the absolute number of kernels. Usually the change is one of merely two or three kernels per ear, as the following figures show. One mutant, however, falls quite outside the frame. (This is the partially sterile mutant 44/32 mentioned in the foot-note):

| | Decrease | | | | | | | | Increase | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Change in kernel number | 7— | 6— | 5— | 4— | 3— | 2— | 1— | 0 | —1 | —2 | —3 | —4 | —5 | —6 | —7 | |
| Number of cases | *1* | — | — | 1 | 5 | 2 | 10 | | 6 | 5 | 2 | — | — | — | — | = 32 |

The eleven mutants that showed the D/m values of 3,0 and more deviated on an average by three kernels per ear from the mother-lines.

[1] If one of the Maja mutants, namely (44/32), which is partially sterile is left out of account, the agreement will be still better, viz. 100 : 110.

Although the direct sterility has only been determined on eight of these 32 vital mutants, it can be unhesitatingly asserted that the great majority have a fertility equal to that of the maternal line, only one or two per cent of the flowers failing. There can be no question of any strong gamete or embryo lethality.

In the summer of 1945 *the number of spikelets* (florets) per ear in some thirty mutants was determined by direct counts. The mutants studied were such as had been included for one or several years in the yield trials, and were thus more or less valuable from a practical point of view.

| | Lower number of florets | | | | | | Higher number of florets | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Change in number of florets | 5 | 4 | 3 | 2 | 1 | 0 | 1 | 2 | 3 | 4 | 5 | |
| Number of cases | — | — | 3 | 1 | 11 | 9 | 4 | — | — | *1* | | = 29 |

Only one mutant (44/9 from Golden barley) differs notably from the mother-line. The difference is significant in this case (D/m = 6,3). In other respects this mutant deviates by its pale-green tone of colour, broad leaves, increased culm- and ear-length and its lateness. Otherwise the Golden barley type has been closely kept.

Seventeen mutants, which were examined both as regards kernel number (1944) and number of spikelets (1945), showed a positive, significant correlation between the two properties. This confirms the view that the raised (or reduced) kernel number is mainly due to the fact that the number of spikelets has risen (or fallen).

The *tillering capacity* of a commercial strain, as in a mutant, has a great influence on the yield. Twenty-two mutants out of Maja barley showed in 1944 the following D/m values for the differences from the mother-line (25—75 plants taken at random):

| | Lower tillering capacity | | | | Higher tillering capacity | | | | |
|---|---|---|---|---|---|---|---|---|---|
| D/m | — 3 | — 2 | — 1 | — 0 | — 1 | — 2 | — 3 | — | |
| Number of cases | — | 3 | 1 | 4 | 4 | 7 | 2 | *1* | = 22 |

One mutant falls entirely outside the variation of its mother-line (44/89). Probably some of the variants that have D/m values between 2 and 3 are also genetically distinct from the maternal line. On page 37 it was pointed out that every eleventh mutant has an increased kernel number, counted per ear. According to the above group of figures, every *twenty-second* mutant has a significantly richer tillering

capacity. No mutant has yet been observed that combines positive deviations (by D/m > 3) in both the characters. But the mutant 44/89 just mentioned will probably prove on closer examination to be so constituted, the difference from the maternal line amounting in tillering capacity to $1{,}00 \pm 0{,}33$ (D/m = 3,03) and in the number of kernels per ear to $0{,}98 \pm 0{,}37$ (D/m = 2,65). In three cases (*erectoides* 12 and 14 as well as 44/98) the tillering as well as the kernel number was reduced. The yield for *erectoides* 12, however, ranks level with that of the Maja barley itself.

As regards the *germinability of the seeds* it may be said in general that this is not reduced in any of the mutants that have been tested in yield trials. At the sowing in 1945 the germinability was 99 % in seven specially examined morphological mutants, and likewise 99 % in twelve physiological mutants. The two maternal lines Golden barley and Maja barley showed 98 %. In the laboratory experiments of 1945, especially arranged to elucidate the germinating ability, the germination figures for the same mutants were 97—100 %. Several mutants show a retarded germination ripeness in certain years (p. 36). But this change is of an enzymatic nature and does not signify any lethality.

In his paper of 1926 HALLQVIST showed that dihybrids between different chlorophyll mutants often have disturbed segregation numbers in the $F_2$, although female gametes and zygotes are only exceptionally lethal. If germinability is lowered, the fall is usually quite inconsiderable (in the most pronounced dihybrid case 6,6 %; monohybrid and constantly green plants possessed a zygote lethality of 2,2 %). The same applies to the seed-setting (in the same dihybrid 5,2 % empty florets occurred against 1,7 % in the control plants). This synthetic lethality constitutes the lowest degree of sterility.

A similar form of synthetic lethality, or rather synthetic sterility, arises if different induced erectoid mutants are crossed with one another. The erectoid character (dense, compact ears) is recessive in relation to the nutans type (sparse, slack ears). Monohybrids usually segregate in the proportion of 3 : 1. Dihybrids give in the $F_2$, unless linkage occurs, 9 normals : 6 *erectoides* (single recessives) : 1 *zeocriton* (double recessive). In monohybrids the fertility falls only slightly, whereas in dihybrids it shows a steep fall. This behaviour has been clarified with particular care for three erectoid mutants, all of which had arisen from Golden barley.

| | | | | | | |
|---|---|---|---|---|---|---|
| Golden barley | P-fertility | 97,0 % | 25 | plants, | 25 | ears |
| *Erectoides* 1 | » | 98,0 % | 25 | » | 25 | » |
| *Erectoides* 6 | » | 96,2 % | 25 | » | 25 | » |
| *Erectoides* 7 | » | 98,7 % | 25 | » | 25 | » |
| *Erectoides* 1 × Golden barley | $F_1$-fertility almost normal, not accurately analysed | | | | | |
| *Erectoides* 6 × Golden barley | » | 91,6 % | 20 | plants, | 80 | ears |
| *Erectoides* 7 × Golden barley | » | 89,9 % | 16 | » | 79 | » |
| *Erectoides* 1 × *erect.* 6 | » | 80,7 % | 8 | » | 31 | » |
| *Erectoides* 6 × *erect.* 1 | » | 79,2 % | 16 | » | 68 | » |
| *Erectoides* 1 × *erect.* 7 | » | 59,4 % | 12 | » | 44 | » |
| *Erectoides* 7 × *erect.* 1 | » | 55,0 % | 29 | » | 129 | » |
| *Erectoides* 6 × *erect.* 7 | » | 68,6 % | 10 | » | 48 | » |
| *Erectoides* 7 × *erect.* 6 | » | 72,9 % | 7 | » | 61 | » |

The crosses with Golden barley give a distinctly even if weakly reduced fertility (6 or 7 % below the mother-line). The sterility is far more conspicuous in crosses between the erectoid types themselves, especially in the cross *erectoides* 1 × *erectoides* 7 and reciprocally. In all cases the reciprocal crosses behave identically. No maternal effect is thus seen. These results suggest that the irradiation together with the distinct »gene-change» that results in the erectoid type has also brought about other changes in the genome, e. g. minor inversions and translocations. In homozygous condition these structural re-arrangements exercise no lethal effects and therefore probably do not constitute deficiencies. In heterozygous state — after crossing with the original line — they reduce the fertility somewhat, presumably owing to small meiotic disturbances. If such aberrations are brought together from different mutants independent of one another, their effect is added and the sterility increases considerably. According to this explanation, the 50 % sterility of *erectoides* 1 × *erectoides* 7 is not due to a single reciprocal translocation but to an accumulated effect of genomic re-arrangements. This behaviour resembles what MÜNTZING (1938) has demonstrated for line crosses within *Galeopsis*.

The pronounced pleiotropic effect of the morphological mutants probably depends in several cases on the interaction between an altered gene, often with strong effect, and a number of modifiers, which in their turn may be genic mutations but are probably often associated with structural re-arrangements (position or »pattern» effects, etc.). X-raying strikes and changes the cell nucleus at many points. But complex changes may also arise in association with spontaneous mutations. The additive effect that these show at crosses is certainly less

than that of the X-ray mutations but is nevertheless unmistakable in several cases.

## IV. MUTATION FREQUENCIES IN BARLEY.

### 1. SPONTANEOUS MATERIAL.

Hardly any exact data on the frequency of spontaneous barley mutations are to be met with in the literature. HALLQVIST (1924) found eight plant progenies of about six thousand to be heterozygous for various chlorophyll mutants. His material mainly consisted of progenies from crosses. In pure lines of Scandinavian commercial varieties, e. g. Golden barley, NILSSON-EHLE (unpubl.) has estimated that a new spontaneous chlorophyll mutation appears in the progeny from about ten thousand plants. PETO (1937), on inspection of field material, found 149 mutants among 1.390.000 normal plants (i. e. about one in 10.000). Since the mid-thirties the author has made notes on the progenies from all X-rayed seed as well as on all the material planted as controls. Several spontaneous chlorophyll mutants have been observed during the period covered.

In Golden barley progenies from 8.279 individual plants were specially studied. At least four plants were heterozygous for spontaneous mutations, that is 4,8 per 10.000. No chlorophyll homozygote was observed in the first generation.

In Maja barley two plants out of 2.256 proved to be heterozygous for chlorophyll mutations, i. e. 8,9 per 10.000.

In Ymer $b_7$ barley, finally, one heterozygote was found among 707 offspring, 14,1 per 10.000. (During a field examination in 1944 one *albina* recessive was observed among 4.600 seedlings.)

The different mutant types (*albina*, *alboviridis*, *viridis*, etc.) arise spontaneously in about the same proportions as after X-ray treatment. In X-ray experiments the *albina* mutants usually compose 50—60 % of all mutants. Of twelve spontaneous mutants, five (42 %) were *albina*, two (17 %) were *alboviridis*, and five (42 %) were *viridis* types.

Some experiments have been carried out with the object of clarifying the frequencies of mutations in heat-treated and aged seed material. In series from 1939, where seed material from Golden barley consisting of dry as well as of water-soaked seed had been gradually given a temperature of 80° C. for 24 hours, three to four mutants appeared in 473 progenies. This represents a heterozygote frequency of

63—85 per 10.000, i. e. an increase fifteen times that shown by the normal material. PETO (l. c.) also found a substantial increase.

In 1939 plants were also raised from aged seed (stored with a 15 % water-content, material from 1932—1935). The progenies from 697 such plants contained five mutants, i. e. 72 heterozygotes per 10.000 plants or a fifteen-fold increase in the number of heterozygotes.

The following table shows the results obtained:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Golden barley, pure line ..... | 4 | heterozygotes | in | 8279 | plants, | 4,8 | per 10.000 |
| Maja barley, old cross variety ..... | 2 | » | » | 2256 | » | 8,9 | » » |
| Ymer barley $b_7$, new cross variety .. | 1 | » | » | 707 | » | 14,1 | » » |
| HALLQVIST, 1924, cross material .... | 8 | » | » | 6000 | » | 13,3 | » » |
| Golden barley, heat-treated seed .... | 3 | » | » | 473 | » | 63,6 | » » |
| Golden barley, aged seed ..... | 5 | » | » | 697 | » | 71,7 | » » |

The total frequency of chlorophyll mutants under normal conditions corresponds to the effect in the $X_2$ of 40 *r*-units (Golden barley), 70 *r* (Maja barley) and 110 *r* (Ymer $b_7$ barley). Heat-treated and aged seed give mutant frequencies corresponding to about 500 *r*.

## 2. INDUCED MUTATIONS.

*Chlorophyll mutation.* — The number of chlorophyll mutants formed in different cereals after X-ray treatment was first determined by STADLER. In his work of 1929 he puts the mutation frequency in barley at $4{,}9 \cdot 10^{-6}$, in the diploid *Avena brevis* and *strigosa* at $4{,}1 \cdot 10^{-6}$ and $2{,}6 \cdot 10^{-6}$, and in the diploid *Triticum monococcum* at $10{,}4 \cdot 10^{-6}$, all calculated per *r* unit and *ear* progeny in the $X_2$ generation.

In 1940 GUSTAFSSON reported the frequencies for different mutation types in barley. Calculated per ear progeny the total mutation frequency in the $X_2$ generation is about $4 \cdot 10^{-6}$ for every *r* unit.

FREISLEBEN and LEIN (1943 b) gave a series of valuable data, likewise derived from $X_2$ analyses. They find that the mutation frequencies do not rise proportionally to the amount of X-rays (Fig. 9). In the most extensive series (from the year 1941) the absolute frequencies increase up to a dose of 8.000 to 10.000 *r* in order to fall thereafter. If the frequencies are calculated per *r* unit, they are found to fall continuously from $18{,}4 \cdot 10^{-6}$ at 4.000 *r* to $5{,}6 \cdot 10^{-6}$ at 14.000 *r*. In the second series (from 1942) the absolute frequencies increase rapidly up to 15.000 *r* but then fall again. Calculated per *r*, the values lie at

about the same height. In both cases the frequencies have been calculated per *plant* progeny [1].

The number of *albina* and *xantha* mutants in Maja barley has been determined in the $X_3$ generation after irradiation with six doses between 2.500 and 25.000 *r* (1941—1942). Just as in FREISLEBEN and

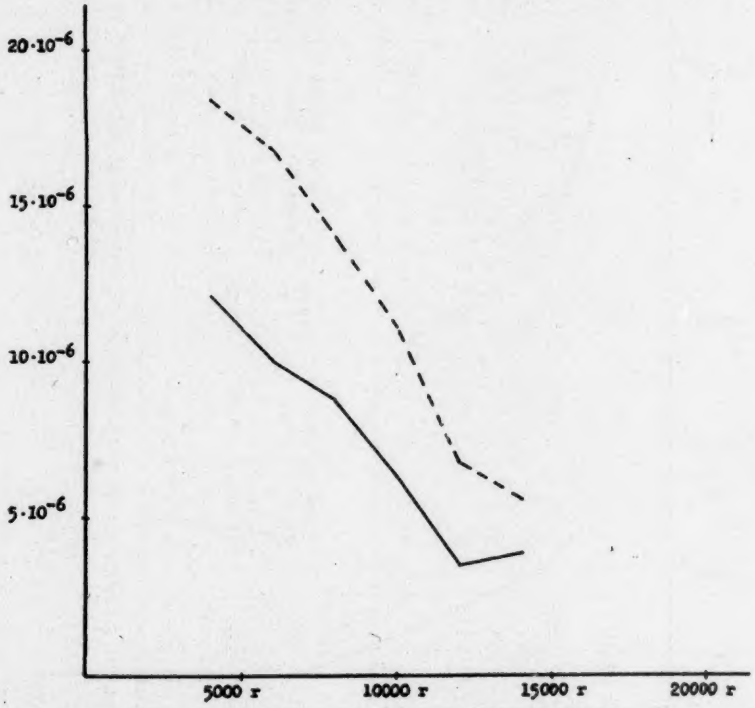



Fig. 9. The frequency of *albina* mutants, calculated per *r*-unit and plant progeny (lower curve). The upper curve shows the frequency of all chlorophyll mutants, calculated per *r*-unit and plant progeny. $X_2$-material. (After FREISLEBEN und LEIN, 1943 b, series 1941.)

LEIN's first series, there was a steep fall in the mutation frequencies per *r* unit with increased X-ray dose (Fig. 10). On an average the $X_1$ plants have developed three to four ears per plant, and hence the frequencies can also be calculated per ear progeny.

[1] The two authors cultivated their $X_1$ plants under very favourable conditions. The plants therefore tillered copiously, giving up to 15 ears per plant.

During the years 1943—1944 a third analysis was carried out, this time in Ymer barley $b_7$ (10.000—25.000 *r*). Only one ear (the principal ear) from each $X_1$ plant was sown. The mutation frequency, calculated per *r* unit, falls successively and reaches its minimum at 25.000 *r*, the same result, accordingly, as was obtained in Maja.

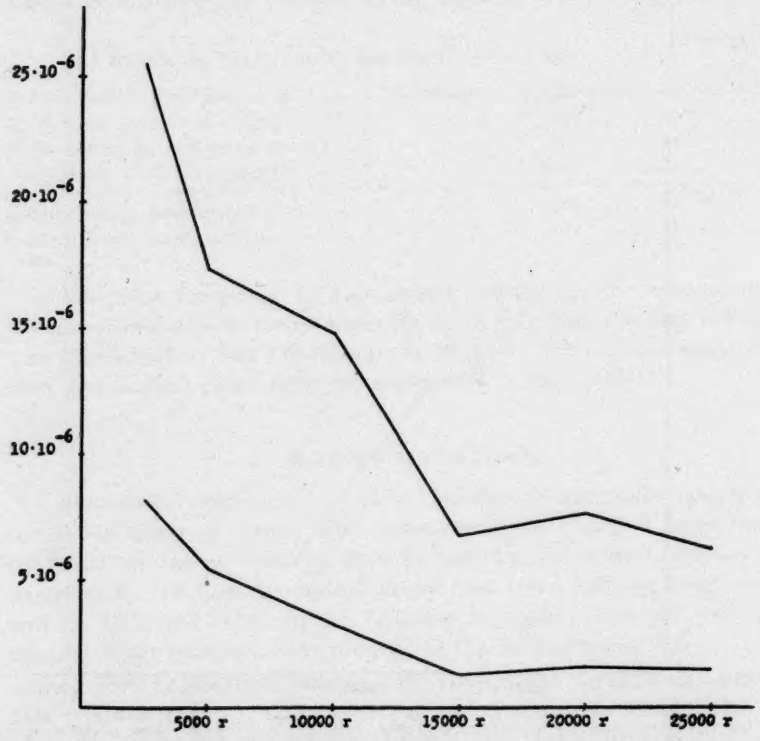



Fig. 10. The frequency of *albina* mutants in Maja barley, calculated per *r*-unit and plant progeny (upper curve), per *r*-unit and ear progeny (lower curve). $X_3$-material.

The absence of proportionality between dose and mutation frequency may be due, as FREISLEBEN and LEIN also point out, to different concurrent circumstances, viz. (1) a saturation effect, that is to say, at high doses a certain mutation type arises several times without its being possible to distinguish the different mutation cases from one another, (2) an S-shaped dose curve; certain mutations and mutation types are not one-hit phenomena but derived from structural re-arrangements, (3) selective conditions during ontogenesis, the most lethal changes being eliminated in favour of undamaged chromosomes and genes, in which connexion the fact also

TABLE 4. *The mutation frequencies of various types of chlorophyll mutations in barley.*

| | Plant progenies | | | | | Ear progenies | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Freisleben and Lein, 1943 | | Gustafsson, 1940 | Maja barley | Ymer barley | Gustafsson, 1940 | Maja barley | Ymer barley | Ymer barley |
| | I. 4.000—14.000 *r* $X_2$ | II. 8.000—20.000 *r* $X_2$ | 5.000—10.000 *r* $X_2$ | 2.500—25.000 *r* $X_3$ | 10.000 *r* $X_3$ | 5.000—10.000 *r* $X_2$ | 2.500—25.000 *r* $X_3$ | 10.000—25.000 *r* $X_3$ | 10.000 *r* $X_3$ |
| *Albina* | $7{,}4 \cdot 10^{-6}$ | $7{,}7 \cdot 10^{-6}$ | $6{,}2 \cdot 10^{-6}$ | $13{,}0 \cdot 10^{-6}$ | $12{,}0 \cdot 10^{-6}$ | $\pm 2{,}1 \cdot 10^{-6}$ | $3{,}4 \cdot 10^{-6}$ | $4{,}6 \cdot 10^{-6}$ | $4{,}0 \cdot 10^{-6}$ |
| *Xantha* | 0,8 | 0,5 | 0,9 | 3,2 | 2,0 | ± 0,3 | 0,8 | 0,2 | 0,7 |
| *Alboviridis* | 1,4 | 1,0 | 3,0 | — | 5,0 | ± 1,0 | — | 0,9 | 1,7 |
| *Viridis* | 2,6 | 5,4 | 2,3 | — | 9,0 | ± 0,8 | — | 1,2 | 3,0 |
| All mutations | 12,1 | 14,7 | 12,7 | — | 30,0 | ± 4,2 | — | 7,2 | 10,0 |
| N | 802 | 936 | 103 | 139 | 30 | 103 | 139 | 90 | 30 |

(All values calculated per *r*-unit.)

has its effect that small seeds are more liable to be killed than large ones and the latter give lower mutation frequencies.

According to the summary given in Table 4, the total frequency of chlorophyll mutations in the $X_2$ generation can be calculated at $\pm 13 . 10^{-6}$ per X-ray unit and plant progeny. The corresponding value in the $X_3$ generation will be $\pm 30 . 10^{-6}$. Thus, in those cases in which the analysis is made in the $X_2$ only one-half of the actual number of mutations are found, the other mutations remaining in heterozygous form. Calculated per ear-progeny the frequencies in the $X_2$ and $X_3$ will be about one-third to one-fourth of the corresponding values for the plant progenies. The agreement between the different experimental series is remarkably good.

The influence of metabolism on the mutation process was first investigated by STADLER (1928 b, 1930). He found about a fourfold increase. In the experiments carried out at Svalöf the frequencies do not rise so steeply, but in any case they double or treble (GUSTAFSSON, 1940). On the other hand, the mutation frequencies fall to about one-half if the seeds are desiccated gradually at 80° C. The results obtained from three X-rayed series are given in the following table. More than 300 $X_2$ mutants have been observed in these experiments.

| | 80° | 10 % $H_2O$ | 15 % $H_2O$ | 23 hours $H_2O$ | 0,01 % heteroauxin |
|---|---|---|---|---|---|
| Golden barley, all mutations | — | 10,2 | 15,4 | 21,7 | 26,3 |
| Maja barley, *albina* ..... | — | 6,5 | — | 15,5 | — |
| Ymer barley, *albina*..... | 3,7 | 8,0 | — | — | — |

$. 10^{-6}$

(All figures calculated per *r* unit and plant progeny.)

*Viable chlorophyll mutants.* — Besides chlorophyll mutants that die at seedling stage, there also arise now and again ones that are able to go on living and produce germinable seed year after year. Their seedlings are often pale-green to yellowish-green, and the fully grown plants have a paler tone of colour than what is normally the case. In these mutants earing and ripening are generally considerably delayed. For the most part they belong to the *viridis* group. A few viable *alboviridis* and *tigrina* mutants have been obtained.

In an $X_3$ generation of Maja barley, where the $X_1$ seed had been X-rayed with 2.500—25.000 *r*, there arose eleven *viridis* mutants, one *tigrina*-like *viridis* mutant and one *alboviridis* mutant, all of which

were homozygously viable. The average frequency calculated per *r* unit and plant progeny amounts to $1{,}3 \cdot 10^{-6}$, calculated per *r* unit and ear progeny it amounts to $0{,}3 \cdot 10^{-6}$. About every twenty-fifth chlorophyll mutant is thus viable in the homozygous state.

*Sterility aberrants.* — Sterile individuals often appear in the progeny of the $X_1$ plants. The sterility is due partly to structural heterozygosity (chromosomal sterility, according to DOBZHANSKY), partly to recessive factors (deficiencies, genic mutations) that in homozygous form prevent sex-cells from developing or functioning (genic sterility). The two sterility types can be distinguished from each other to a certain extent, the meiosis-disturbing factors often bringing about complete sterility. On the other hand, translocation sterility in barley reduces the fertility by 20—50 %.

A preliminary analysis of the sterility behaviour of the X-rayed Ymer $b_7$ barley has been performed. The material originates from seed treated with 20.000 and 25.000 *r*. $X_2$ progenies were raised from one ear of each $X_1$ plant. The $X_2$ generation was classified into (1) progenies consisting of completely fertile or slightly sterile plants, (2) progenies with one or more rather sterile plants (fertility between 75—30 %), (3) progenies with solitary highly sterile plants (fertility about 10 %) and, finally, (4) progenies with solitary completely sterile plants. Group 1 contains unchanged plants as well as plants with small translocations, inversions, deficiencies in heterozygous form. Group 2 contains pronounced structural heterozygotes. Groups 3 and 4 comprise recessive mutations with a changed floral structure, dwarf growth, asynapsis, polymitosis, stickiness and so on as a consequence.

In all, progenies from 558 $X_1$ ears were planted. Of them, 84 gave one or more rather sterile plants (group 2: 15 %), 11 contained one or more very sterile plants (group 3: 2 %), and 17 gave solitary completely sterile plants (group 4: 3 %). The frequency is $6 \cdot 10^{-6}$ per *r* for group 2 and $3 \cdot 10^{-6}$ for groups 3 and 4 combined. »Meiosis-destroying» mutations thus occur in the $X_2$ to about as numerous an extent as all chlorophyll mutations taken together. The gross structural re-arrangements that reduce the fertility considerably appear more often.

The segregation in groups 3 and 4 should approach 3 : 1 (the chimaeric structure of the $X_1$ ears causes a deficiency of recessives), in group 2, on the other hand, more likely 1 : 1 (here, too, with a deficiency for the sterile plants). In group 3 the segregation was 99 : 27, in group 4 it was 188 : 64, i. e. in both cases within the limits of a simple Mendelian segregation. In group 2 the analysis gave 734 completely

or almost completely fertile plants to 319 rather sterile ones. The D/m according to the 3 : 1 scheme is 4,0 (with a considerable surplus of sterile plants). Hence the sterility in group 2 probably depends to a great extent on translocation heterozygosis.

As sterile and lethal mutants have only been cursorily studied, this analysis cannot be considered as final.

*Vital mutants.* — A most important question from a general point of view is that of the frequency of the vital mutants. In five series

TABLE 5. *Number of vital mutations in three different barley strains.*

| Strain | Treatment | Plant progenies | | Chlorophyll mutations ($X_2$) | Morphological mutations ($X_2$) | | Physiological mutations ($X_2$) | |
|---|---|---|---|---|---|---|---|---|
| | | No. of plants | No. of ears | | | | | |
| Ymer I | 10.000 *r* Changed physiol. ..... | 802 | (2137) | ± 12 % | 11 = | 1,4 % | ? | |
| Maja I | 2.500—25.000 *r* | 1030 | (4359) | ± 14 % | 4 = | 0,4 % | 11 = | 1,1 % |
| Maja II | 5.000—10.000 *r* Changed physiol. ..... | 1226 | (5322) | ± 10 % | 3 = | 0,2 % | 5 = | 0,4 % |
| | | Ear progenies | | | | | | |
| Ymer II | 10.000–25.000 *r* | (1208) | 1208 | ± 4 % | ≧ 6 = | 0,5 % | ? | |
| Golden I | 5.000—10.000 *r* Changed physiol. ..... | (1768) | 1768 | ± 5 % | ≧ 2 = | 0,1 % | ≧ 2 = | 0,1 % |
| Golden II | Changed physiol. ..... | (862) | 862 | ± 7 % | 1 = | 0,1 % | ≧ 2 = | 0,2 % |

of barley grown 1939—1945 and treated with exactly determined X-ray doses, not only the number of chlorophyll mutants but also the number of morphological vital mutants and to some extent that of the physiological vitals were determined (principally in the $X_2$ generation). In three series (Ymer $b_7$ 1, Maja 1 and Maja 2) field progenies were reared from all the ears of the $X_1$ plants, in three others (Ymer $b_7$ 2, Golden barley 1 and 2) the different progenies originated from one ear (the principal ear) of each $X_1$ plant. No physiological variants have been recorded from Ymer barley $b_7$, which represents a strain recently produced.

Ymer $b_7$ gave a high number of morphological mutants in series 1 (10.000 *r*, different pre-treatment of the original seed). Against ± 100

chlorophyll mutations in the $X_2$ there are *at least* eleven morphological mutations, seven of which are erectoid types, two bright-green types, one calcaroid, one *bracteatum*. This means that every tenth mutation of those observed in the $X_2$ is morphological and vital.

In Ymer 2, where the original seed had been given X-ray doses from (500—)10.000 to 25.000 *r*, 52 cases of chlorophyll mutants were observed against at least six morphological vital types, viz. *erectoides* 17—19 (20), a short-awned erectoid type, one bright-green variant as well as one *intermedium* mutant. Thus, in this series too, every tenth $X_2$ mutant is morphological and vital.

Maja 1, where the original seed had been given from 2.500 up to 25.000 *r*, shows a lower mutation frequency. Against a calculated number of 154 chlorophyll mutants there are only four morphological and vital types, namely *erectoides* 12—15. Only about every fortieth mutation distinctly observable in the $X_2$ is thus morphological and vital.

In Maja 2, where the seeds pretreated in different ways had been given 5.000 and 10.000 *r*, there arose 62 *albinas* in the $X_2$. This figure corresponds to about 125 chlorophyll mutants. The three morphological and vital mutants consisted of *erectoides* 16 and two bright-green variants. Here too, therefore, every fortieth $X_2$ mutant deviates morphologically and is vital.

Golden barley 1 (cf. GUSTAFSSON, 1940, the 1939 series: 17—24) produced in the $X_2$ generation 92 chlorophyll mutants and two (to three) vital morphological mutants (*erectoides* 9 and 10, possibly also a *tetrastichum* variant). About every fiftieth mutant is thus vital and morphological.

The series named Golden barley 2 in Table 5 originates from seeds which had been treated in different ways before being X-rayed. The dose was not measured in *r* units. A single vital morphological type (*erectoides* 11) was observed. In addition, 63 chlorophyll mutants were formed. Hence, in this series every sixtieth $X_2$ mutant is vital and simultaneously morphologically changed.

The three barley strains thus give vital morphological mutants in the proportion of 1 in 10, 1 in 40, 1 in 52, the average being 1 in 23. Ymer $b_7$ seems most liable to produce morphological mutants, Golden barley least liable, Maja having about a mid-position. The different capacity of the three strains to produce morphological mutants corresponds to different mutation frequencies in spontaneous material. As was earlier remarked, Golden barley represents a very old pure line

(isolated before 1900), while Maja and Ymer are recent cross-products with Golden barley as one of the original types. Ymer barley is the most complex as well as the last formed product.

Several physiological mutants have been isolated in the $X_2$ and $X_3$ of the Maja and Golden-barley series. They have often been observed already in the $X_2$, but the range of modification of normal material is so wide that their nature of mutations cannot always be established until in the $X_3$. Individual progenies in great numbers should preferably be raised in the $X_3$ and $X_4$ and then searchingly compared with the maternal line. This is however possible only to a limited extent. The mutants observed are therefore only a fraction of those that have actually been formed. Unless special precautionary measures are taken (p. 51), spontaneous crossings and solitary seed intermixtures may sometimes be classed as mutants. Physiological variants cannot be awarded the same general value as evidence as morphological ones. In the six series which have been most thoroughly examined, and in which the sources of error have been successively eliminated, at least twenty cases of completely fertile physiological mutations have been observed (Table 5).

In Maja 1 eleven physiological mutants correspond to about 154 chlorophyll mutants, i.e. a proportion of 1: 14. In Maja 2 five physiological mutants arose. The proportion in this case ($\pm$ 125 chlorophyll mutants) is 1: 25. In Golden barley 1 two physiological mutants were isolated as against 92 chlorophyll aberrants. This means a proportion of 1: 46. Finally, in Golden barley 2, two or probably three physiological types were observed as against 63 chlorophyll mutants, i.e. a ratio of 1: 32.

The Maja barley seems more liable to give physiological mutants than the Golden barley, *which as a rule behaves very stably.* On an average the four series gave about two vital physiological mutants as against one morphological.

Of the morphological mutants enumerated here, eleven have up to now been tested in yield trials (p. 60). Four of them have given about the same yield as the mother-line, one has shown a better result.

All the fertile mutants produced in the physiological group have been tested in respect of their yield. Of the twenty types, nine are equal to and two probably superior to the maternal lines, i.e. show about the same ratio as within the morphological group.

## V. THE MODE OF ORIGIN OF INDUCED MUTANTS.

### 1. SOURCES OF ERROR AND METHODS OF CONTROL.

The control of the mutants and their mode of origin have been sharpened as the investigations have advanced. However, even in the predominantly self-fertilizing varieties of cereals — barley, oats and wheat — spontaneous crossing occurs to a varying extent in different lines (STEVENSON, 1928). The risk of crossings can nevertheless be eliminated by means of suitable experimental conditions. Another source of error consists in contamination of seed material or admixture of foreign seed. An admixture is however rather easy to discover, as in such cases the progenies from all the ears deviate in a similar manner from the control.

The following precautions have been successively adopted. (1) The seed material to be X-rayed is taken from élite material of a pure line, often from individual plants, which also have to supply control series. If a non-homogeneous commercial strain is to be X-rayed, only morphological aberrants are isolated. (2) The $X_1$ (as well as the $X_2$) generation should as far as possible be grown apart from other field material of the same variety of plant. (3) Each $X_1$ plant is harvested and threshed separately and its kernels are sown separately ear for ear. (4) The variants that arise are scrupulously compared with other lines and strains in the assortment. (5) In specially important model experiments use is made of lines that are recessive for certain easily observable properties. For instance, seed of Pudel wheat functioned in the first X-raying experiments in wheat. This line is homozygous for white kernel colour and glume hairiness. Other lines having these properties are not in culture. The kernel colour of the observed variants show whether crossings or mutations have occurred. (6) An extensive control material is sown.

In two-rowed barley the individual control has been carried so far that every mutant arising can be referred not only to a definite $X_1$ plant but also to a definite $X_1$ ear, in which the number of well-developed kernels, as well as the number of empty flowers, is exactly known. In six-rowed barley, wheat and oats it takes up far too much time to determine the exact sterility ear for ear. However, even these kinds of plants have been subjected to as exacting control as possible, especially by way of extensive cultivation of progenies from non-irradiated plants.

It may be added that so careful a testing as this is hardly necessary

if only morphological variants are to be produced. As a matter of fact, so far as these are concerned it is easy to determine whether they originate from mutation, spontaneous crossing or admixture. On the other hand, physiological variants must be scrupulously followed and controlled, since many of them differ only slightly from the maternal line.

As previously mentioned, admixtures can be readily unmasked by the fact that all the ear progenies then deviate. *Individual* ear progenies, on the other hand, may be homozygous for a recessive mutation already in the $X_2$. Some examples may illustrate this. The kernels from three ears of an $X_1$ plant out of Ymer $b_7$ were sown. Seven of nine kernels from ear 2 germinated and all were »*albina*». The two remaining ear progenies were normally green. In another case an $X_1$ ear with eight kernels germinated. The six seedlings that arose represented the rare mutation *alboxantha*. In a third case three ear progenies from an $X_1$ plant were sown. From one of the ears there arose eight identical seedlings of a yellow tone of colour (chlorophyll mutation) and homozygously viable, although greatly delayed in their earing. At times ears that are homozygous for a recessive character (e. g. *albina* or *xantha*) can already be observed on the $X_1$ plant.

Some examples will illustrate the genesis of the mutations:

*Erectoides* 12 arose out of Maja barley. From 865 X-rayed seeds (2.500 *r*) 557 $X_1$ plants were obtained with an average fertility of 89 %. The $X_1$ plant in question showed 95 % fertility, the mutated ear 91 %. The plant possessed nine ears. In the progeny from ear 4 two erectoid plants were observed. Of five selected $X_2$ plants three were normal in the $X_3$, one segregated for *erectoides*, one was a constant *erectoides*.

*Erectoides* 12 deviates not only from Maja barley but also distinctly from all barley lines under cultivation. The $X_1$ generation in question was kept separate. The extensive $X_2$ generation was sown later than the other barley material and apart from this.

*Erectoides* 16 likewise arose out of Maja. The seed material was soaked in water for 21 hours prior to being X-rayed (10.000 *r*). From 1436 sown seeds only 46 $X_1$ plants were harvested, with an average fertility of 66 %. The $X_1$ plant concerned gave twelve ears and possessed 71 % fertility. Fifteen kernels in ear 6 (65 % fertility) gave an $X_2$ generation consisting of seven plants. One of these was recorded as »early ripening». New progenies were raised from five of these seven $X_2$ plants. Three plants were normal, one heterozygous and one homozygous for *erectoides* 16. Seed material from the two remaining $X_2$ plants was sown in the spring of 1945. One was heterozygous for the mutation in question, one did not differ from the mother-line.

The same $X_1$ plant and the same $X_1$ ear gave rise to a physiological mutant (44/109) that deviates from the maternal line, principally by broader leaves. *Erectoides* 16 differs in shape of ear as well as by its extreme earliness. Neither

variant can have arisen as a consequence of spontaneous crossing or seed admixture. Besides them, a *lutescens* mutant was also formed in the $X_2$ progeny from ear 11. The $X_2$ plants from ear 5 were completely sterile.

The cultivating conditions for the $X_1$ and $X_2$ generations were the same as for *erectoides* 12.

An example of a fully controlled still more complex behaviour is offered by a Golden barley series that was already X-rayed in 1937 (1937: 110, GUSTAFSSON, 1940). The treatment was so intensive that from 508 sown seeds there arose only thirty $X_1$ plants, with an average fertility of 32 %. The $X_1$ plant in question possessed a fertility of 37 %. Out of it there arose, *inter alia*, a very broad-leafed »winter-barley like» mutant (44/65, cf. p. 31, difference for leaf-breadth $3{,}6 \pm 0{,}4$ mm.). The same ear progeny gave a physiological variant that differed from Golden barley by somewhat broader leaves (44/23, p. 33, difference for leaf-breadth $1{,}6 \pm 0{,}2$ mm.). In another ear progeny there appeared an *albina* mutant and at the same time a straw-stiff mutant (42/13). The winter-barley like mutant appeared in two of the eleven ears. (There are three to four ear initials in one barley embryo: hence, several of the ears that arose on the harvested plants were secondary and could therefore give the same mutant.)

## 2. $X_1$ FERTILITY AND TYPE OF MUTATION.

As the fertility of the maternal plant is known for most of the induced mutants, it is possible to determine the X-ray effect required to induce a certain mutation or mutation type. To take an example from Ymer $b_7$ barley: The mutant »short-awned» arose from an $X_1$ plant with only 42 % fertility, *erectoides* 22 from an $X_1$ plant with 92 % fertility. This decrease in fertility illustrates the nuclear changes required to enable the two mutants to arise (if, of course, these fertility figures are representative). »Short-awned» thus requires for its origin a greater change in the genotypic constitution of the $X_1$ seed in question than *erectoides* 22.

Another example may be appended to this: GUSTAFSSON (1940) considered that he was able to determine that *viridis* mutations preferably arise in the progeny of sterile $X_1$ plants while the *albina* mutations are readily formed in the progeny of fully fertile plants also. The former would thus occur predominantly in fertility classes in which translocations, inversions, etc. have accumulated. (This view has been criticized by MÜNTZING, 1942, as well as by FREISLEBEN and LEIN, 1943; see GUSTAFSSON, 1946 b.) In the mutation experiments of the last five years 471 *albina* mutants have been produced. The corresponding $X_1$ plants possessed an average fertility of $77{,}0 \pm 0{,}7$ %. The *viridis* mutants, numbering 136, showed an average $X_1$ fertility of $71{,}4 \pm 1{,}4$ %. The difference is statistically significant. Hence, for their origin the

*viridis* mutants need between 5 and 6 % lower average fertility in the $X_1$ plants than the *albina* types.

The following series is obtained:

| Mutation type | *albina* | *xantha* | *alboviridis* |
|---|---|---|---|
| Average $X_1$-fertility: ..... | 77,02 ± 0,68 % | 74,09 ± 1,38 % | 74,07 ± 1,47 % |
| Number of mutants: ..... | 471 | 66 | 108 |

| *viridis* | physiological mutants | morphological mutants |
|---|---|---|
| 71,40 ± 1,43 % | 71,03 ± 3,72 % | 67,50 ± 3,34 % |
| 136 | 29 | 36 |

According to these data chlorophyll mutants, especially *albinas*, are on an average considerably more liable to arise than physiological and morphological mutants (i.e. result from less drastic changes). The differences however are not significant, except in the case of *albina* and *viridis* types (D/m = 3,6), as well as probably in that of *albina* and morphological mutants (D/m = 2,8). Physiological mutants (most closely comparable with »Kleinmutationen» in BAUR's sense) arise, regarded as a group, more frequently than morphological, although many of them are discovered only after careful $X_3$ analysis, and they also seem to be formed somewhat more readily.

Of far greater interest, however, is the fact that the morphological mutants can be divided into groups that possess distinct sterility properties. One group comprises the bright-green, »non-glaucous» mutants, which have been described on page 29. Another group consists of the erectoid mutants, which are sharply delimited and easy to define (p. 20). To a third group belong the rare mutations which more or less drastically change the phenotype and in several cases cause the spikelets and flowers to get quite a new structure.

The degree of pleiotropism runs roughly parallel with the increased degree of drasticity. Certainly the bright-green mutants deviate sharply from the maternal lines, though only in individual characters. Many of the erectoid types are very pleiotropic (p. 23). This condition is probably due to the fact that gene changes and structural rearrangements have simultaneously occurred. Reasons arguing in favour of this assumption have been submitted earlier. Among the drastic mutants are ranked *calcaroides*, *bracteatum*, *densinodosum* (2 cases), »lemma-like glumes» (2 cases), *intermedium*, *tetrastichum*, short-awned, »winter-barley like» mutants with altered flower structure (2

cases). The spontaneous mutant *scirpoides* ought also to be placed in this group. These three groups show significant differences in plant as well as ear fertility in the $X_1$ generation, as the following table shows (GUSTAFSSON, 1946 a).

| | »Non-glaucous» | *Erectoides* | Drastic mutants |
|---|---|---|---|
| (A) Average fertility of the mutated $X_1$-plants ..... | $78{,}50 \pm 6{,}55$ % | $73{,}00 \pm 2{,}71$ % | $51{,}27 \pm 6{,}07$ % |
| (B) Average fertility of the mutated $X_1$-ears ..... | $92{,}75 \pm 3{,}92$ % | $74{,}57 \pm 4{,}39$ % | $55{,}82 \pm 7{,}90$ % |
| Number of mutants ..... | 4 | 21 | 11 |

In group (A) the difference between erectoid and drastic mutants is significant ($0{,}01 > P > 0{,}001$), as it also is between the bright-green and the drastic mutants ($0{,}01 > P > 0{,}001$).

In group (B) two differences are significant, between »non-glaucous» and erectoid mutants on one hand ($0{,}01 > P > 0{,}001$) and »non-glaucous» and drastic mutants on the other ($P = 0{,}001$). The difference between erectoid and drastic mutants corresponds to a P value of $0{,}05$.

The fact that the three groups are significantly separated from one another is also shown by an analysis of variance. The quotient inter/intra-class gives a P value which in both (A) and (B) lies between $0{,}01$ and $0{,}001$.

These results may be construed in two ways: *either* the individual genes which, after mutating, drastically alter the phenotype are so stable that they are affected only by the very strongest X-ray action, *or* the drastic morphological changes require, in order to be realized at all, a complete reconstruction of the chromosome material, for instance changes in one principal gene accompanied by drastic re-arrangements in the chromosomes, in modifiers, polygenes and so on.

The yield of the morphological mutants falls in the measure the changes become greater. Three tested bright-green mutants gave an average yield index of 101 (against 100 for the mother-lines). Fourteen erectoid types gave the relative value 88. Of the drastic mutants, only two have been tested so far (»lemma-like glumes» 1 and 2). These gave a yield index of 84. Of the rest, the *densinodosi* are completely sterile. *Calcaroides* is vegetatively distinctly weaker than the maternal line. In *bracteatum* the ears bend just before ripening and hang down, a clearly negative character. The two »winter-barley like mutants» give a low yield of poorly ripened kernels when grown as spring barley; grown as winter barley they are unable to endure the winter. *Scirpoides*, the only spontaneous drastic mutant, is distinctly

weakened, with smaller ears and fewer kernels. Hence the drastic mutants — at any rate in the area of cultivation of the original line itself — give a substantially lower yield and are in some cases non-vital.

An interesting fact is that the yield within the erectoid group also falls in the measure the deviations from the original line are accentuated. In the following table the yield, straw-length and ear-length of the maternal lines have been put at 100.

| Yield | Slightly changed ear- and straw-length; kernel character unchanged | Greatly reduced ear-length (rel. fig. $< 80$) | Greatly changed straw-length (rel. fig. $< 90$ or $> 110$) | Greatly changed ear- and straw-length | Changed kernel characters |
|---|---|---|---|---|---|
| Golden barley mutants ..... | 101, 97, 91 | 92, 91 | *83* | — | *89, 83* |
| Maja barley mutants ..... | 95 | 97 | 100 | *76, 51* [1] | — |

### 3. EXPERIMENTAL CONTROL OF THE MUTATION PROCESS.

The important problem of whether the mutation process can be experimentally controlled has in recent years been made the subject of special investigations. The results are as yet meagre but seem to go in a positive direction. RHOADES's important investigations of 1938 and 1945 pointed clearly in this direction. Before that, DEMEREC (1937) had observed that a definite gene in the second chromosome of *Drosophila melanogaster* was able to increase the mutability of the whole genome very considerably. In RHOADES's case in maize the labilizing effect proved to be specific. The mutability was increased solely in a definite gene. The gene $a_1$ is very stable in the presence of gene *dt* and instable in company with the gene *Dt*. Other genes modify the effect of *Dt*. Chromosome aberrations do not occur.

In 1940 GERSHENSON observed that *Drosophila* larvae fed with the sodium salt of thymonucleic acid — whether followed by X-raying or not — give predominantly mutants that affect the structure of the wings. This result has been contended by RAPOPORT (1940). The

[1] *Erectoides* 14 with the relative figure 76 has also changed kernel character, *erectoides* 15 with the figure 51 has uncoloured nerves on the lemmas.

same year GUSTAFSSON considered he was able to show that the so-called *alboxantha* mutation in barley (see below) arises in considerably higher frequency if the seeds are soaked in water or heteroauxin before being X-rayed. The next year (1941) DOTTERWEICH and SCHMIDTKE believed they were able to prove that, if *D. melanogaster* is fed with follicle hormone before irradiation, the visible mutants increase in number and change character as well. Mutations that alter the type of the wing are replaced by such as effect the bristles and the venation of the wings. With very high quantities of hormone the stability of the genome is considerably reduced. The visible mutants increase many-foldly as compared with the spontaneous cultures. Bristle mutants dominate. Especially the sex-linked gene *scute* and the autosomal gene *Stubble* mutate. Genes having definite phenotypic effect but located in different chromosomes are thus labilized by a change in the external environment.

The *alboxantha* mutation in barley is very rare both as a spontaneous and an induced product. Probably it is due to changes in a definite gene (this is not yet proved). The material collected since 1940 corroborates earlier observations, viz. that *alboxantha* mutants are considerably more liable to arise from seeds soaked in water and auxin than from normally and artificially dried seed material.

In laboratory experiments (the only reliable method) with Golden barley and Maja barley eleven *alboxantha* mutants arose, which were distributed over different seed materials as follows:

| | Dry seeds contain previous to irradiation | | Dry seeds previous to irradiation pretreated with | |
|---|---|---|---|---|
| | 10 % $H_2O$ | 15 % $H_2O$ | $H_2O$ 23 hours | Auxin 23 hours |
| Number of $X_1$-progenies ..... | 2184 | 586 = 2770 | 501 | 702 = 1203 |
| » » chlorophyll mutants | 254 | 66 = 320 | 109 | 128 = 237 |
| » » *alboxanthas* ..... | 0 | 1 = 1 | 3—4 | 6 = 9—10 |
| » » *tigrinas* ..... | 7 | 3 = 10 | 1 | 0 = 1 |

The distribution dry *contra* soaked series gives $P = 0{,}001$ for *alboxantha* as against non-*alboxantha* mutants (according to YATE's correction $0{,}01 > P > 0{,}001$), while for *alboxantha*-mutated as against non-*alboxantha*-mutated $X_1$ plants it gives $P < 0{,}001$ (before as well as after correction for low number of mutants).

In the progenies from Ymer $b_7$ (chiefly dry seed with 12 % $H_2O$

which had received 500—25.000 *r*) not a single *alboxantha* mutant arose in the indoor material. On the other hand, in the field experiments, which do not allow of a fully reliable classification, *possibly* one *alboxantha* mutant was formed (after 25.000 *r*). Dry seed from Maja barley did not yield any *alboxantha* even after the highest doses (25.000 *r*).

This indicates that the cell-nuclei must be in a certain physiological state for *alboxantha* mutations to arise. If the high hydration is not present, even the highest X-ray doses are unable to induce them.

Another mutation type, *tigrina*, likewise very rare, shows in the same material a contrary state of things (see above). The difference as against *alboxantha* is statistically significant ($P < 0{,}001$, with or without YATE's correction). It has arisen five times from Ymer $b_7$, in all cases in dry series.

The identity of the various *alboxantha* mutants has admittedly not yet been cleared up, but even if several genes should be responsible for one and the same phenotypical effect, most of them must respond in quite a definite manner to changes in the state of the cells.

Another example may be submitted to show that mutations do not arise quite at random. *Xantha* mutants, as they are defined and classified by me, show a tendency to accumulate in fertility regions having a slightly but distinctly reduced fertility (70—90 %) and to avoid regions with full fertility (90—100 %) or low fertility (0—70 %) (GUSTAFSSON, 1940, 1946 b). On an average they show no significant average decrease in the fertility of the $X_1$ plants as compared with the *albina* types (see the table on p. 54), but the distributions are entirely different:

| | Fertility of the $X_1$ plants | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0—10 | — 20 | — 30 | — 40 | — 50 | — 60 | — 70 | — 80 | — 90 | — 100 % |
| *Albina* ..... | 1 | 2 | 5 | 18 | 36 | 66 | 115 | 133 | 95 | = 471 |
| *Xantha* ..... | | | | 2 | 3 | 13 | 20 | 25 | 3 | = 66 |

The difference in distribution is statistically significant (in a $2 \times 2$-table P will be greater than 0,001 but less than 0,01, in a $2 \times 5$-table P is 0,01).

An analysis of the distribution of *albina* and *xantha* mutants in relation to the number of $X_1$ plants in the different fertility classes shows that the *albina* types are regularly distributed over the whole of the area, while the *xantha* types accumulate in regions with a reduced fertility. Two of the three *xantha* mutants, the mother-plants

of which showed 90—100 % fertility, arose from plants with 91 % fertility, i.e. values below those of the control plants (96—97 %). The mother-plant of the third *xantha* mutant certainly possessed 96 % fertility, but in the same ear progeny there also arose one *albina* and one *alboviridis* mutant. In spite of the apparently normal fertility, therefore, a very strong X-ray effect must have occurred.

There is hardly any doubt that the *xantha* mutations — whatever their innermost nature may be — generally require a more powerful X-ray effect than the *albina* mutations; they cannot arise in the progeny of completely fertile $X_1$ plants. To obtain *xanthas* it is necessary to induce sterility and raise progenies from distinctly but weakly sterile plants.

Judging from these results, therefore, the mutation process does not proceed at random; it is pre-determined, can be controlled by altering the *cell environment* in a definite manner. RHOADES's exceedingly important investigation, already cited earlier in this work, shows that individual genes are stabilized or labilized when the gene environment is changed.

Experiments are in progress on a large scale to endeavour to direct the mutation process methodically so far as vital mutants are concerned. As was mentioned earlier (p. 55), the three groups of morphological mutants show quite different sterility dependence.

## VI. YIELD AND QUALITY.

### 1. THE YIELD OF THE INDUCED MUTANTS.

#### A. SVALÖF TRIALS.

The yield of the barley mutants has been determined by trials spread over several years at the barley department of the Swedish Seed Association. During the first three years these trials were being conducted (1940—1942) this department was under the direction of Prof. H. NILSSON-EHLE, subsequently (1943—1945) under that of Dr. I. GRANHALL. The department's assistants Mr. C. G. VON SYDOW and Mr. C. BERGSTRÖM were responsible for the practical parts of trials during the same period.

The yield trials were laid down in the following way:

1940 — Preliminary series, see GUSTAFSSON, 1941 b, p. 344.

1941 — Two series. A. Machine-sown trials, plots $7 \times 1{,}36$ m. ($9{,}52$ m$^2$), four replications, 200, 300, 400 and 200 kg of saltpetre per hectare. B. Hand-sown trials (= 1940), four replications, same manuring as in A.

1942 — A. Machine-sown trials, plots 6,5 × 1,36 m. (8,84 m²), four replications, manuring as in 1941. B. Hand-sown trials (= 1940) but only three replications: 200, 300, 400 kg saltpetre.

1943 — A. Machine-sown trials, plots 6,5 × 1,36 m. (8,84 m²), four replications, manuring as in 1941. B. Hand-sown trials (= 1940), three replications.

1944 — A. Machine-sown trials, plots 6 × 1,58 m. (9,48 m²). Three replications. Manuring 200, 300 and 400 kg saltpetre per hectare. B. Small machine-sown trial, plots 4 × 0,75 m. (3 m²), three replications: 200, 300, 400 kg saltpetre.

1945 — A. Machine-sown trials, plots 7 × 1,28 m. (8,96 m²), three replications, manuring 200, 300, 500 kg saltpetre. Mutants out of Golden barley. B. Same as A. Mutants out of Maja barley. C. Machine-sown trial, plots 3,7 × 1,28 m. (4,74 m²), two replicates, 200 kg nitrate of lime. Mutants out of Golden barley. D. Same as C. Mutants out of Maja barley. E. Same as C. Mutants out of Maja barley. F. As for C but only one plot of each number. Mutants from Maja and Ymer barley.

Mutants showing reduced fertility were not included in the yield trials, nor were mutants that are fertile but vegetatively weak or abnormal (dwarfs, too late variants, etc.). A strong selection for rich tillering, for general viability and for fertility had already been carried out in the segregating $X_2$ and $X_3$ generations.

The sowing and harvesting were effected by the barley department and carried out by the standard methods applied by the Swedish Seed Association.

In the years 1943—1945 yield trials were also laid down at some of the branch stations of the Seed Association.

The mutual relation of the standards (the mother-lines) as well as their absolute yield has some significance in this connexion. In six years of mutation experiments Maja barley has been on an average 12 % over Golden barley (lowest 7 % in 1943, highest 21 % in 1942). Maja barley averaged 4740 kg per hectare in the machine-sown trials of 1941—1945. The official trials for Malmöhus län, the administrative district in which Svalöf is situated, show for the six-year period 1938—1943 a superiority by 10 % for Maja barley and for the same strain an absolute yield of 4140 kg per hectare. There is thus rather good agreement between the Svalöf figures and the official ones. Mutants out of Ymer $b_7$ were only tested one year (1945). Ymer barley $b_7$ itself gave the relative value of 97 in comparison with Maja barley. In the Seed Association's yield report for Ymer barley (strain $b_1$; GRANHALL, 1944) a relative figure of 99 is given.

### a. Morphological mutants.

In the course of years nineteen vital morphological mutants have been tested for their yield. As shown by the following survey, the mutants have on an average produced yields 90 % of that of the maternal lines. Seven mutants stand about level with the mother-line or higher.

These are naturally the only ones of any great interest in this connexion.

| | Yield (mother-line = 100) | | | | | | | Average |
|---|---|---|---|---|---|---|---|---|
| | 45 | 55 | 65 | 75 | 85 | 95 | 105 | 115 | | |
| Golden mutants ..... | | | 3 | 5 | 2 | | | | = 10 | 89,5 |
| Maja mutants ..... | 1 | | 1 | 1 | 4 | | | | = 7 | 88,0 |
| Ymer mutants ..... | | | | 1 | | | 1 | | = 2 | (98,0) |
| Total ..... | 1 | | 4 | 7 | 6 | | 1 | | = 19 | 90,0 ± 3,0 |

Respecting *erectoides* 1 (Table 6) there are now so many data as to warrant the definite assertion that it is as highly yielding as the mother-line. It is further distinctly straw-stiffer (for the three years 1943—1945 graded at 7,4 against 5,7 for the mother-line). The 1000 grain weight and hectolitre weight are about the same as in the mother-line, and so are the protein and starch contents. The ripening time is slightly longer (one or two days).

*Erectoides* 7 is a little inferior in respect of yield. Its straw-strength is however higher (the same as in the preceding case). It has a somewhat lower 1000-grain weight and hectolitre weight, and ripens distinctly later (two or three days).

*Erectoides* 12 out of Maja barley has naturally enough a considerably higher yield than the two preceding mutants and is fully on a level with the mother-line. Its straw-strength is better (8,8 against 8,0), its 1000-grain weight and hectolitre weight about the same, likewise its earliness (possibly one day later).

*Erectoides* 16, which showed a poor result in the trials of 1944 but a considerably better one in those of 1945, is the most interesting of the morphological mutants from a practical point of view, especially on account of its pronounced pleiotropism. Its yield lies a little under that of the mother-line, but this is undoubtedly due to its extreme earliness (six or seven days). Its 1000-grain weight is distinctly higher (41,9 against 38,5 grams), and so is its straw-strength (8,8 against 7,9). In 1945 it was also tested in northern Sweden, where its earliness may have some value. It has been crossed on a large scale with the earliest commercial strains.

*Erectoides* 13 is especially interesting on account of its extreme straw-strength (9,9 against 7,5). The hectolitre weight is higher but the 1000-grain weight somewhat lower than the mother-line's. It yields somewhat less.

The two bright-green mutants, which judging from the figures

rank level with or above the mother-line in yield, have not as yet been tested sufficiently long for a reliable opinion to be given. Mutant No. 3 was included in a small trial. Two different pedigree lines, however, were tested and gave the relative values 110,0 and 101,7. In other qualities the two mutants stand close to the mother-lines.

### b. Physiological mutants.

Thirty-one vital mutants have been tested in yield trials in the course of years, fifteen of them originating from Golden barley, six-

TABLE 6. *The yield of the seven best morphological mutants in barley.* (Parents = 100.)

| Variety | 1940 | 1941 | 1942 | 1943 | 1944 | 1945 | | Average |
|---|---|---|---|---|---|---|---|---|
| Golden barley | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Erectoides* 1 | 101,3A | 100,2A | 101,3A | 93,6A | 102,5A | 102,3A | 104,9C | 100,9 |
| *Erectoides* 7 | | | 92,9A | 96,7A | 100,4A | 97,7A | 100,0C | 97,5 |
| Maja barley | | | | | 100 | 100 | 100 | 100 |
| *Erectoides* 12 | | | | | 99,5A | 101,3B | 99,2D | 100,0 |
| *Erectoides* 13 | | | | | 93,2A | 101,0B | | 97,1 |
| *Erectoides* 16 | | | | | 85,9A | 100,5B | 99,5E | 95,3 |
| Bright green 2 | | | | | 95,8B | 108,8B | | 102,3 |
| Ymer barley $b_7$ | | | | | | 100 | | 100 |
| Bright green 3 : ped. 1 | | | | | | 110,0F | | 105,9 |
| ped. 2 | | | | | | 101,7F | | |

(The letters behind the yield figures denote the experiment series of the year in question. Cf. p. 60.)

teen from Maja barley. The average yield amounts to 94 %, i.e. a little higher than for the morphological group. The difference is not significant, however.

| | Yield (mother-line = 100) | | | | | | M |
|---|---|---|---|---|---|---|---|
| | 65 — 75 — 85 — 95 — 105 — 115 | | | | | | |
| Golden mutants | 1 | 1 | 7 | 5 | 1 | = 15 | 93,0 |
| Maja mutants | | 3 | 4 | 7 | 2 | = 16 | 94,9 |
| Total | 1 | 4 | 11 | 12 | 3 | = 31 | 93,9 ± 1,1 |

Fifteen mutants are about equal to or better than their mother-lines (Table 7). Of the three mutants with relative values between 105 and 115, one (44/7) was tested in three annual yield trials, and

TABLE 7. *The yield of the fifteen best physiological mutants in barley.* (Parents = 100.)

| Variety | Characteristics | 1940 | 1941 | 1942 | 1943 | 1944 | 1945 | Average |
|---|---|---|---|---|---|---|---|---|
| *Golden barley* | | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 44/2 | Late, tall. ..... | $108{,}2_A$ | $110{,}0_A$ | $88{,}7_A$ | $99{,}2_A$ | $109{,}3_A$ | $101{,}8_A$ | 102,9 |
| 44/9 | Broad-leafed, late ... | | | | $100{,}5_A$ | $104{,}7_A$ | $99{,}5_A$ | 101,6 |
| 44/7 | Large seeds ..... | | | | $101{,}6_A$ | $111{,}3_A$ | $113{,}7_A$ | 108,9 |
| 44/23 | Broad-leafed ..... | | | $100{,}7_B$ | $98{,}0_B$ | $101{,}9_B$ | $103{,}3_A$ | 101,0 |
| 44/14 | Straw-stiff, early | | | | | | | |
| | ped. 1 ..... | | | | $105{,}1_B$ | $103{,}4_A$ | $103{,}6_A$ | 103,8 |
| | ped. 2 ..... | | | | $110{,}3_B$ | $97{,}8_A$ | $102{,}5_A$ | |
| 44/25 | Tall, stiff | | | | | | | |
| | ped. 1 ..... | | | | $101{,}7_B$ | $97{,}6_B$ | | 98,3 |
| | ped. 2 ..... | | | | $95{,}1_B$ | $98{,}7_B$ | | |
| *Maja barley* | | | | | | 100 | 100 | 100 |
| 44/18 | Straw-stiff, early ..... | | | | | $94{,}1_A$ | $106{,}5_B$ | 100,3 |
| 44/30 | Seeds differently coloured ..... | | | | | $103{,}0_B$ | $102{,}8_B$ | 102,9 |
| 44/31 | Broad-leafed, late ... | | | | | $100{,}2_B$ | $98{,}0_B$ | 99,1 |
| 44/88 | Early ..... | | | | | | $98{,}6_D$ | 98,6 |
| 44/76 | Waxy | | | | | | | |
| | ped. 1 ..... | | | | | | $102{,}8_D$ | 108,5 |
| | ped. 2 ..... | | | | | | $114{,}1_F$ | |
| 44/85 | Tall, long ears | | | | | | | |
| | ped. 1 ..... | | | | | | $93{,}8_D$ | 95,1 |
| | ped. 2 ..... | | | | | | $96{,}3_D$ | |
| 44/89 | Short | | | | | | | |
| | ped. 1 ..... | | | | | | $114{,}3_D$ | |
| | ped. 2 ..... | | | | | | $109{,}5_D$ | 109,6 |
| | ped. 3 ..... | | | | | | $105{,}1_D$ | |
| 44/91 | Tall, seeds differently coloured | | | | | | | |
| | ped. 1 ..... | | | | | | $106{,}7_D$ | 103,2 |
| | ped. 2 ..... | | | | | | $99{,}7_F$ | |
| 44/100 | Short | | | | | | | |
| | ped. 1 ..... | | | | | | $98{,}7_E$ | |
| | ped. 2 ..... | | | | | | $94{,}9_E$ | 96,2 |
| | ped. 3 ..... | | | | | | $94{,}9_E$ | |

hence the mean value of these should be rather reliable. It deviates especially by its large kernels (the 1000-grain weight for the years 1943—1945 was 43,2 as against 39,7 for the mother-line), its broad

leaves, short straw and at the same time higher straw-strength (6,3 against 5,3), and is somewhat earlier than the maternal line (one day).

44/89 is an apparently high-yielding mutant out of Maja barley, which it much resembles. It has a shorter culm, somewhat inferior straw-strength but as against this it is about three days earlier in ripening. Three different pedigree lines of this mutant were tested in 1945, each in two replications. All were substantially above Maja in yield.

44/76 has a deviating kernel colour and is more waxy than Maja. It was tested in two series in 1945. The relative value is not so reliable as in the preceding case. Its 1000-grain weight stands somewhat higher. The ripening time has been shortened a little (one day).

Of the remaining twelve special mention may be made of the following.

Much like Golden barley are 44/2 and 44/23. The former, a »late, tall» mutant, has been tested for six years and given the relative value of 103. In 1945 it was ten centimetres taller than the mother-line and five days later in ripening. Its straw-strength seems to be good in spite of the high culm (1943—1945: 6,2 against 5,3 for the mother-line). It is characterized by very great fluctuations in yield. These are probably connected with the variation in precipitation during the vegetation period. Following the relatively dry summers of 1940, 1941 and 1944 the yield was at 108, 110 and 109. During 1942, 1943, 1945 with their rich rainfall it was at 89, 99 and 102 (see below). In the year 1942 there was a heavy rainfall just at the time the mutant was earing (fertilizing time), with much lodging as a result.

| | 1940 | 1941 | 1944 | 1943 | 1945 | 1942 |
|---|---|---|---|---|---|---|
| Precipitation from sowing to harvest, mm. ..... | 135 | 173 | 206 | 225 | 256 | 265 |
| Relative yield ..... | 108,2 | 110,0 | 109,3 | 99,2 | 101,8 | 88,7 |

44/23, a segregate out of the same $X_1$ ear as gave the »winter-barley like» mutant 2, deviates from Golden barley by its somewhat broader leaves (p. 32). Its 1000-grain weight is likewise a little higher (43,2 against 38,5 grams). It seems to be one or two days later in ripening.

Compared with the mother-lines, two mutants are distinctly straw-stiffer, 44/14 (8,1 against 6,2) and 44/30 (8,9 against 7,2). Three weaker in straw are 44/25, 44/88 and 44/91. Substantially earlier (up to four days) are 44/18, 44/85 and 44/88. The last-mentioned is also characterized by high straw, long ears and high 1000-grain weight (44,1

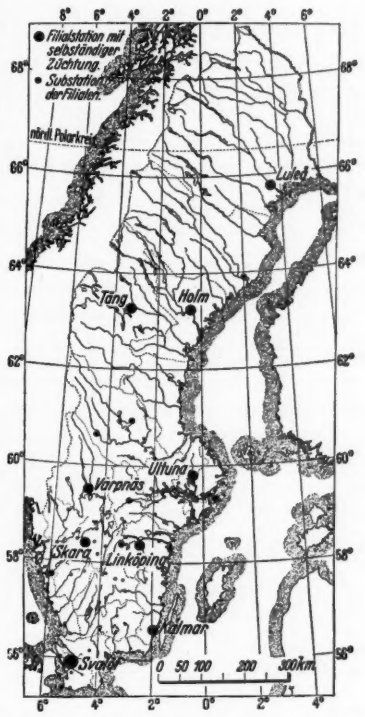
against 37,0). Other valuable mutants listed in Table 6 fluctuate round the mother-lines in earliness, straw-strength, 1000-grain weight, straw-height, ear-length.

In addition to these thirty-one mutants, some variants have been included in one or more annual yield trials. As it cannot be definitely proved that they have arisen as induced mutations, they are not included in the survey on page 62. One of them, 44/3, an extremely waxy, bushy and peculiar variant, originated from Golden barley, was discussed on p. 33. It deviates considerably from all barley lines at Svalöf. Besides the above-mentioned two qualities it is characterized by extreme straw-strength (9,3 against 6,2). Unfortunately, it possesses a low 1000-grain weight (33,7 against 39,7), to a large extent owing to the awns being shed at an early stage. The yield was:

| 1942 | 1943 | 1944 | 1945 | Average |
|---|---|---|---|---|
| 108,8 | 94,8 | 100,2 | 104,6 | 102,1 |

B. TRIALS AT THE BRANCH STATIONS.

The system of branch stations of the Swedish Seed Association takes in the whole country (see Map 1). In recent years certain mutants have been tested in regular trials at the Kalmar station, Ultuna station, Västernorrland station (Länäs, close to Holm), and the Luleå station. The results are only to be regarded as preliminary. The trials will be repeated and extended.




Map 1. The stations of the Swedish Seed Association.

*Kalmar station.* — In the year 1945 the trials were stationed at the Ekerum farm in the island of Öland opposite the town of Kalmar. The district is characterized by severe summer drought and a dry, calciferous, rather improductive soil. Five Golden barley mutants were tested. The plots were 20 $m^2$ in size with four replicates of each number. For Golden barley the crop per hectare amounted to 3025 kg. A land variety peculiar to Öland (Löt) gave 2965 kg. The figures were:

| | Yield | 1000-grain weight | Ripening time, days |
|---|---|---|---|
| Golden barley | 100 | 36,7 | 102 |
| *Erectoides* 1 | 106,8 | 36,5 | 102 |
| 44/2 | 101,5 | 36,0 | 103 |
| 44/3 | 98,5 | 33,9 | 100 |
| 44/7 | 120,1 | 40,4 | 100 |
| 44/14 | 109,9 | 36,9 | 100 |
| Land barley | 98,0 | 38,9 | 101 |

The high yield of *erectoides* 1, the large-kernelled mutant (44/7) and the upright early mutant (44/14) may be specially noted. The 1000-grain weight for 44/7 is very high, whereas it is low for 44/3.

*Ultuna station.* — This branch is distinguished by severe drought in early summer. Four mutants out of Golden barley were compared in 1944 and 1945 with their mother-line, likewise four mutants out of Maja barley in 1945. The size of the plot was 7,5 $m^2$ the first year, 10 $m^2$ the second year, and the number of replicates was four. For Golden barley the hectare yield in 1944 amounted to 2925 kg (manuring 200 kg superphosphate per hectare) and in 1945 to 3805 kg (manuring as in 1944); for Maja barley it amounted in 1945 to 4230 kg. In 1944 the drought was particularly severe. The results were:

| | Yield 1944 | Yield 1945 | Average | Straw-strength | 1000-grain weight | Ripening time, days |
|---|---|---|---|---|---|---|
| Golden barley | 100 | 100 | 100 | 3,3 | 45,1 | 101 |
| *Erectoides* 1 | 88,3 | 100,1 | 94,2 | 4,4 | 44,5 | 99,5 |
| 44/2 | 113,9 | 102,1 | 108,0 | 2,4 | 46,7 | 105 |
| 44/3 | 90,9 | 97,5 | 94,2 | 9,1 | 41,1 | 98,5 |
| 44/7 | 95,2 | 113,5 | 104,4 | 8,5 | 47,3 | 101 |
| Maja barley | | 100 | | 3,9 | 45,1 | 104 |
| *Erectoides* 12 | | 99,2 | | 8,6 | 45,5 | 104 |
| » 13 | | 95,5 | | 10,0 | 43,6 | 104 |
| » 16 | | 91,3 | | 10,0 | 46,3 | 103 |
| 44/18 | | 98,5 | | 8,6 | 45,9 | 103 |

The late, tall mutant 44/2 seems especially adapted to the conditions at this station. A notable feature is the high yield during the dry summer of 1944 (p. 64). The large-kernelled mutant 44/7, out of Golden barley, attained a higher yield in 1945 than Maja barley, but in 1944 did not come even up to the level of Golden barley. Two Maja mutants are very close to their mother in yield (*erectoides* 12, 44/18).

The high straw-strength in some of the mutants is of considerable

interest. In the year 1945 there were heavy falls of rain, which caused serious lodging. Exceptionally straw-stiff were two erectoid mutants out of Maja barley, these being given the highest possible value, 10, as against only 3,9 for the mother-line, as well as the waxy peculiar mutant out of Golden barley, 44/3, with the relative value 9,1 against 3,3 for the maternal line. Two physiological mutants 44/7 and 44/18 surpassed even the parents.

*Västernorrland station.* — This station is the plant-breeding centre of central Norrland and is as a general rule characterized by a short, relatively dry summer (though much rain fell in 1945). Yield trials have been laid down since 1943.

1943: Preliminary trials. Plots only 1 m² in size, one plot of each number. Three Golden-barley mutants were tested, viz. *erectoides* 1, waxy 44/3 and a deep golden-yellow chlorophyll aberrant 42/325, which in two annual yield trials at Svalöf gave 10 % below the mother-line.

1944: This year large machine-sown series were laid down. A. *Erectoides* 1, 44/3 and »late, tall» 44/2 were tested. The plot size was 13,95 m² and the number of replicates four. The manuring consisted of 50 kg of superphosphate, 50 kg of potash and 150 kg of saltpetre per hectare. B. The three mutants of the 1943 trials were sown in preliminary series. Plot size: 13,95 m², one plot of each. Manuring: 100 kg of superphosphate and 100 kg of potash per hectare.

1945: Three machine-sown series. A. Four mutants out of Golden barley. Plot size, 11,5 m². Four replicates. Manuring 50 kg. of superphosphate, 50 kg of potash, 50 kg of saltpetre per hectare. B. Four mutants out of Maja barley. Plot size 7,5 m², four replicates. Manuring as for preceding. C. Two mutants out of Golden barley, material grown at the branch since 1943. One plot of each, 13,5 m².

The yield for Golden barley was 4430 kg/hectare in 1944, 3830 kg in 1945; for Maja barley 4530 kg in 1945. In normal cases the maternal varieties are too late for this area and consequently unsuitable for cultivation on a large scale.

| | Yield | | | | | | Straw-strength | 1000-grain weight | Ripening time, days |
|---|---|---|---|---|---|---|---|---|---|
| | 1943 | 1944 | | 1945 | | Average 1944 + 1945 | | | |
| | | A | B | A + B | C | | | | |
| Golden barley.. | (100) | 100 | 100 | 100 | 100 | 100 | 2,3 | 43,2 | 90 |
| *Erectoides* 1 .. | (106,9) | 100,7 | 119,7 | 97,4 | 100,0 | 104,5 | 4,4 | 40,8 | 89 |
| 44/3 ..... | (111,5) | 104,7 | 111,8 | 100,5 | 90,6 | 101,9 | 7,9 | 40,2 | 87 |
| 44/2 ..... | | 104,7 | | 103,7 | | 104,2 | 1,5 | 43,8 | 96 |
| Maja barley .. | | | | 100 | | | 1,3 | 45,0 | 93 |
| *Erectoides* 12.. | | | | 98,2 | | | 1,4 | 45,7 | 94 |
| » 13.. | | | | 96,9 | | | 3,1 | 43,6 | 93 |
| » 16.. | | | | 93,2 | | | 3,0 | 48,4 | 89 |
| 44/18 ..... | | | | 95,4 | | | 1,8 | 46,6 | 93 |

All three Golden-barley mutants showed up better than the maternal line in respect of yield. Especially valuable is the high straw-strength of *erectoides* 1 and 44/3 accompanied as it is by earlier ripening (and earing). The late mutant 44/2 attains a strong vegetative development at the branch station and becomes lodged to a serious extent.

The previously mentioned golden-bright chlorophyll aberrant 42/325 out of Golden barley deserves special mention here. At Svalöf it gives a low yield (10 % below Golden barley). At Lännäs it gave a relative figure of 119,5 in 1943 and a yield of 130,3 in 1944, i. e. very high figures. It eared and ripened in 1944 about a week before the mother-line. Unfortunately, uniform seed from the mother-line and the mutant were not sown in the year 1945. Thus, there is no reliable value for 42/325 for that year. The above-cited relative figures are suggestive of an altered physiological adjustment, presumably connected with the long length of the day in the area concerned.

The yield of the Maja mutants is somewhat lower throughout than that of the maternal line. *Erectoides* 16 also combines here great earliness, increased straw-strength and higher 1000-grain weight.

An interesting feature is that both Maja barley and its mutants are weaker in the straw than Golden barley and its mutants, a condition directly opposite that applying in South Sweden. A tendency in the same direction was already observed in the Ultuna material. The waxiness mutant 44/3 is very straw-stiff, in 1945 straw-stiffer than all other material sown in a similar manner.

*Luleå station.* — This branch, lying as it does at 66° N. lat., only 65 (English) miles south of the polar circle, has a short (although rather mild) period of vegetation. As a rule the rainfall is sufficient. The summer of 1945 was unusually long and hence the examined X-ray mutants, as also their mother-lines, were able to set ripe seed. The early six-row barley Vega was included in the trials.

The size of the plot was 6,4 $m^2$ and the number of replicates three. Manuring consisted of 300 kg of potassium nitrate, 300 kg superphosphate and 100 kg potash per hectare. The yield for Golden barley amounted to 2570 kg per hectare, for Maja barley to 3450 kg. The material of Golden barley and its mutants was derived from the 1944 cultivations at Lännäs, the material of Maja barley and its mutants from Svalöf.

Luleå presents a repetition of the results at Lännäs concerning the inverse relation of the straw-strength of Golden barley and Maja

| | Yield | Straw-strength | 1000-grain weight | Ripening time, days |
|---|---|---|---|---|
| Golden barley ..... | 100 | 5,3 | 49,1 | 94 |
| *Erectoides* 1 .... . | 92,2 | 6,7 | 47,1 | 96 |
| 44/3 ..... | 82,9 | 9,0 | 44,2 | 92 |
| Vega barley ..... | 87,5 | 8,0 | 43,4 | 80 |
| Maja barley ..... | 100 | 5,0 | 53,5 | 94 |
| *Erectoides* 12 .... . | 103,2 | 6,7 | 51,7 | 98 |
| » 13 ..... | 91,6 | 7,3 | 47,9 | 91 |
| » 16 ..... | 97,7 | 7,3 | 54,7 | 93 (?) |
| 44/18 ..... | 101,7 | 5,0 | 53,7 | 91 |
| Vega barley ..... . | 88,1 | 8,0 | 45,9 | 79 |

barley as well as their mutants. The waxiness mutant 44/3, in which the earliness is also increased, is the straw-stiffest of all the mutants and substantially straw-stiffer than the maternal lines. Vega barley, however, ripens almost a fortnight before. In yield the Maja mutants show up rather well. The figures however are almost only of curiosity interest.

## 2. SOME QUALITY CHARACTERS OF THE VITAL MUTANTS.

To meet all demands for direct practical value a vital mutant must not only have a high yield but also possess good 1000-grain and hectolitre weights as well as good straw-strength. If a mutant is to be of practical value, it should in addition have, used as feeding barley, a high protein content, used as malt barley, special malting properties.

The content of protein (or starch) has in the course of years been determined for seventeen mutants out of Golden barley and for eight mutants out of Maja barley (crude protein in % of dry matter). Golden barley itself has shown a protein content of 12,3 % as against 10,6 for Maja barley in five annual yield trials. The Golden-barley mutants group themselves round their mother-line, the Maja mutants round theirs. Reduced in proportion to the average value of the mother-lines, the mutants are distributed as follows:

| | 9,45-9,95 | 10,45-10,95 | 11,45-11,95 | 12,45-12,95 | 13,45-13,95 | 14,45 % | M |
|---|---|---|---|---|---|---|---|
| Golden barley | | | X | | | | 12,3 % |
| Mutants | | 1 3 | 4 | 1 1 | 3 2 | 2=17 | 12,3 % |
| Maja barley | | X | | | | | 10,6 % |
| Mutants | 2 2 | 3 — | 1 | | | =8 | 10,5 % |

The above figures show very clearly that distinct group differences exist. No mutants have arisen with a specially high protein content

concomitant with a high yield. Among the Golden-barley mutants the best is then the peculiarly golden-bright mutant 42/325 with a 10 % lower yield at Svalöf but at the same time a protein content of 15,1 % as against 14,0 % for Golden barley (1940—1941). The best among the Maja mutants is *erectoides* 16 with a 5 % lower yield but a protein content of 10,3 % as against 9,3 % (1944).

In the mutant material examined there exists a distinct correlation between high yield and low protein content. Putting the yield of the maternal lines at 100 and their protein content at 10,0, the relative values for the mutants will be:

| Rel. yield | 65 — 75 | — 85 | — 95 | — 105 | — 115 | |
|---|---|---|---|---|---|---|
| Rel. amount of protein ..... | 11,60 | 10,83 | 10,34 | 9,71 | 9,70 | |
| No. of mutants ..... | 1 | 3 | 5 | 15 | 1 | = 25 |

Respecting the starch content there is not much to say. High protein content is usually correlated to low starch content and *vice versa.*

On material from the 1944 and 1945 yield trials the malting quality was determined in eighteen mutants, six of which were morphological mutants and twelve physiological. These mutants have a yield level with or above that of the maternal lines. They represent a one-sided selection from the point of view of quality also. The evaluation was carried out by A. B. Stockholms Bryggerier under the direction of Mr. H. THUNÆUS, Engineer. Mr. THUNÆUS has published a report of the results (1946), which has been taken as a base for this survey.

Each sample was divided into halves and malting tests made on each half. The malt was analysed with regard to the extract content of fine and coarse grist, saccharification, vitreosity, and so on. In addition, determinations were made of the wort nitrogen in percentage of the total nitrogen in the malt, the diastatic power as a measure of the $\beta$-amylase content and the starch liquefying power as a measure of the $\alpha$-amylase content. With the aid of the malting loss and the extract content of the malt the extract production was then determined in percentage of the steeped dry matter of the barley. In a malting barley variety of the first rank — according to the stipulations of the Swedish breweries — the soluble nitrogen must be above 38 % and the fine-coarse difference under 1,1 %. The diastatic power should be higher than 200 units.

TABLE 8. *The malting quality of the eight best mutants in Golden barley.* (After THUNÆUS, 1946.)

| Variety (Cf. Tables 6 and 7) | Protein per cent in dry barley (N × 6,25) | Extract in malt, fine grist, on dry basis | Extract diff. between fine and coarse grist | Wort N per cent of malt nitrogen | Diastatic power in malt on dry basis | Starch liquefying power on dry basis | Extract produced in malt % of barley dry subst. |
|---|---|---|---|---|---|---|---|
| *Erectoides* 1 | 10,4 | 78,1 | 1,5 | 33,7 | 169 | 233 | 71,7 |
| *Erectoides* 7 | 10,7 | 77,7 | 1,0 | 32,1 | 175 | 234 | 71,6 |
| 44/2 | 9,7 | 78,1 | 0,7 | 36,1 | 174 | 239 | 71,6 |
| 44/3 | 10,5 | 77,6 | 1,1 | 33,7 | 145 | 215 | 71,4 |
| 44/7 | 10,4 | 78,0 | 1,3 | 39,1 | 209 | 305 | 71,2 |
| 44/9 | 9,7 | 79,2 | 0,9 | 38,2 | 199 | 286 | 72,7 |
| 44/14 | 9,8 | 78,8 | 1,4 | 33,5 | 203 | 261 | 72,6 |
| 44/23 | 10,4 | 79,0 | 1,1 | 36,8 | 237 | 319 | 72,5 |
| Golden barley | 11,2 | 78,3 | 1,1 | 33,8 | 177 | 245 | 71,3 |
| Significance of variety differences | | | | | | | |
| P | — | 0,001 | — | 0,001 | 0,001 | 0,001 | — |
| Smallest significant difference | — | 0,37 | — | 1,82 | 4,9 | 13,5 | |

TABLE 9. *The malting quality of ten mutants in Maja barley.* (After THUNÆUS, 1946.)

| Variety (Cf. Tables 6 and 7) | Protein per cent in dry barley (N × 6,25) | Extract in malt, fine grist on dry basis | Extract diff. between fine and coarse grist | Wort N per cent of malt nitrogen | Diastatic power in malt on dry basis | Starch liquefying power on dry basis | Extract produced in malt % of barley dry subst. |
|---|---|---|---|---|---|---|---|
| *Series 1.* | | | | | | | |
| *Erectoides* 12 | 9,8 | 78,7 | 1,1 | 36,2 | 271 | 348 | 72,4 |
| *Erectoides* 13 | 9,8 | 78,0 | 1,1 | 36,7 | 287 | 350 | 72,2 |
| *Erectoides* 16 | 9,5 | 78,3 | 0,9 | 38,6 | 261 | 347 | 71,8 |
| Bright green 2 | 9,3 | 78,5 | 1,6 | 36,4 | 255 | 340 | 71,9 |
| 44/18 | 9,9 | 79,2 | 1,3 | 36,8 | 243 | 357 | 73,0 |
| 44/30 | 9,7 | 78,2 | 1,3 | 36,7 | 261 | 334 | 72,2 |
| 44/31 | 10,3 | 77,5 | 1,2 | 36,2 | 306 | 394 | 71,5 |
| Maja barley | 9,4 | 78,5 | 1,3 | 38,5 | 269 | 360 | 72,0 |
| Significance of variety diff. | | | | | | | |
| P | — | 0,05 | — | 0,05 | 0,001 | — | — |
| Smallest significant difference | — | 0,69 | — | 1,57 | 11,4 | — | — |
| *Series 2.* | | | | | | | |
| 44/76 | 9,3 | 80,1 | 1,2 | 39,4 | 284 | 379 | 73,5 |
| 44/89 ped. 1 | 9,5 | 79,5 | 1,0 | 39,2 | 320 | 418 | 72,5 |
| ped. 2 | 9,6 | 79,4 | 0,8 | 39,9 | 313 | 420 | 72,2 |
| ped. 3 | 9,1 | 79,9 | 0,7 | 40,7 | 280 | 377 | 73,2 |
| 44/91 | 9,8 | 79,0 | 0,9 | 39,3 | 160 | 248 | 72,6 |
| Maja barley | 9,0 | 80,2 | 0,9 | 40,2 | 289 | 377 | 73,5 |
| Significance of variety diff. | | | | | | | |
| P | — | 0,05 | — | — | 0,001 | 0,001 | — |
| Smallest significant difference | — | 0,67 | — | — | 28,7 | 25,2 | — |

The figures submitted below have reference only to year 1945.

In the morphological mutants tested (Tables 8 and 9) the malting properties have only been slightly altered, in some cases in a weakly negative direction, in one case in a positive direction. This is the more remarkable as other physiological properties have to some extent been considerably displaced. Probably the tested variants represent the very best both in yield and malting quality. Under all circumstances it is incontestable that morphological mutants may signify an improvement in straw-strength and earliness simultaneously with retention of the yield and kernel quality of the original lines.

The group differences are very distinct, especially as regards the diastatic and the starch liquefying power.

Of the physiological mutants out of Golden barley (Table 8), one (44/3) is signalized by a wretched quality. One mutant (44/2) is practically identical with the mother line, possibly somewhat improved with respect to the quantity of nitrogen in the wort. The mutant 44/14 seems to be slightly better, in any case as regards diastatic power. A decidedly superior mutant in the trial of 1945 is the broad-leafed 44/9, which on account of its lateness and its feeble straw has no immediate practical value.

There remain the two mutants 44/7 and 44/23. Both are distinguished by considerably increased kernel size, the former also by a higher yield. At the same time the malting quality has risen considerably, especially in diastatic power and dextrinizing ability. The values here lie far outside the limits of error. The greatest interest attaches to 44/23. In external qualities this is an image of its mother-line (p. 32). The straw-strength is exactly the same, and so is the yield. It would therefore seem justifiable to assume that the improved malting quality in this case is directly due to the increased seed size. From the same $X_1$ plant there arose, besides this highly interesting type, three worthless mutants, a winter-barley like mutant (44/65), a low-yielding straw-stiff mutant (42/13), and an *albina* mutant.

Golden barley represents an original stage in respect of malting properties. Hydrolyzing enzymes certainly occur rather abundantly in this barley as compared with many other land varieties. By means of crossing and recombination varieties have been produced in which these enzymes are elaborated in far greater quantities. Among these are Maja barley and its still better sister-varieties Kenia and Opal. It is therefore rather probable, as THUNÆUS has suggested, that these strains are less liable to mutate further than Golden barley.

In a Maja mutant (44/91) with long yellowish-brown ears and weak straw the quality has deteriorated disastrously. It falls even below the Golden-barley level so far as diastatic power and dextrinizing ability are concerned (Table 9). Despite this, its 1000-grain weight is enormous (1945: 11 and 12 % higher than that of Maja and Golden barleys) and it yields substantially more than both these strains.

Three mutants (44/18, 44/30, 44/76) are roughly equal in quality, the two first-mentioned being possibly somewhat inferior as regards wort nitrogen and diastatic power.

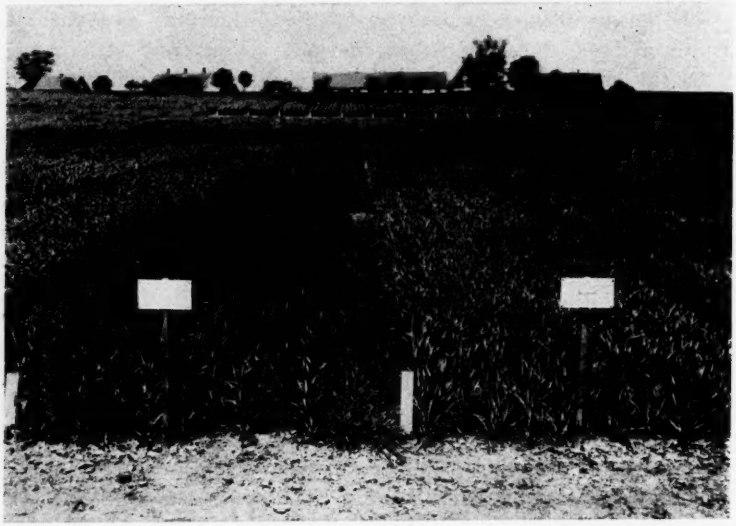


Fig. 11. The extreme difference in earliness between *erectoides* 16 (to the left) and its mother strain Maja barley (to the right). Photographed on June 22, 1946. Yield experiments.

The late mutant 44/31 seems to be definitely superior in regard to diastatic power. Strangely enough it has a low 1000-grain weight. The mutant 44/89 seems to be clearly positive. On an average it gives a 10 % higher yield than Maja (1945) and at the same time shows a distinct improvement of the diastatic power in two of the three pedigrees examined. The 1000-grain weight is almost exactly the same. If these results hold on re-examination, it will be possible, even in so eminent a variety as Maja barley, to induce mutants that improve the quality and simultaneously increase the yield.

The weather conditions affect the malting results in a high degree. The trials reported above must therefore be judged with due caution.

### 3. RELATION BETWEEN THE YIELD OF THE MUTANTS AND THE $X_1$ FERTILITY.

The mitotic injuries increase in frequency and strength in the measure the X-ray irradiation is intensified. At the same time the chromosome breaks increase in number (with translocations and inversions as a consequence), the re-arrangements become grosser, the gene mutations increase. Those seeds which after being X-rayed give rise to the most sterile $X_1$ plants should therefore contain the highest number of deficiencies, inversions and translocations. Gene mutations which cause visible changes in the phenotype are consequently often combined with gross structural re-arrangements, in the same or in other chromosomes [1].

The results seem to show that some connexion exists between the yield of the extracted mutants and the fertility of the corresponding $X_1$ plants. The fewer chromosome aberrations in the irradiated seeds the greater is the general fertility in the $X_1$, $X_2$ and later generations and at the same time the higher is the yield of the isolated mutants.

A distinct even if feeble positive correlation can be demonstrated for the morphological as well as for the physiological mutants, whether they have arisen from Golden barley or from Maja barley. The $X_1$ fertility is known for seventeen morphological and twenty-nine physiological mutants, which have in addition been tested in one or several annual yield trials. For the whole material the regression coefficient ($b$) amounts to 0,58, which implies that the fertility increases by 0,6 % for every one per cent increase in the yield. The correlation coefficient ($r$) is 0,32 and its P value 0,02.

The tested mutants out of Golden barley show a stronger correlation ($r = 0{,}52$, $0{,}02 > P > 0{,}01$). For the Maja mutants $r$ is only 0,11 and $P > 0{,}20$. The low correlation coefficient for the last-mentioned is probably to some extent due to the average fertility having fallen more profoundly in the Golden-barley material than in the Maja series, with a more powerful interference in the genotype as a consequence. But it may also be associated with the fact that the yield figures for the

[1] Theoretically, mutant individuals that are phenotypically identical but genotypically dissimilar may be isolated from a segregating $X_2$ generation. Different pedigree lines can therefore give different yields, depending upon whether they are afflicted with deficiencies and lethals, or are quite free from such.

Maja mutants are not yet entirely reliable. The general course of events is however the same.

Physiological mutants, taken separately, give a P value of 0,05, morphological mutants a P value greater than 0,05. The probable existence of a correlation in the last-mentioned as well is shown by the following survey.

| | Fertility of the $X_1$ plants in percentages | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10 — | 20 — | 30 — | 40 — | 50 — | 60 — | 70 — | 80 — | 90 — | 100 |
| Yield for Golden mutants, Golden = 100 ..... | *80* | | *83* | *89* | *89* | *83*, 92 | | 91, 98 | | |
| Yield for Maja mutants, Maja = 100 ..... | | | | | | | *51*, *76*, 95 | 95, 102 | 97, 100 | |

The occurrence of a correlation is further confirmed if the mutants are divided into two groups, one comprising mutants equivalent to or better than the maternal lines (relative values 95—115) and the other containing mutants that are inferior to the mothers (relative values below 95), and the average $X_1$ fertility is then determined for each group.

| | | Average $X_1$ fertility | D/m | n | P |
|---|---|---|---|---|---|
| Morphological mutants | Equivalent or better | 84,00 ± 4,15 % | 2,33 | 6 | $0{,}05 > P > 0{,}02$ |
| » » | Inferior ..... | 66,65 ± 6,20 % | | 11 | |
| Physiological mutants | Equivalent or better | 76,93 ± 4,32 % | 1,61 | 14 | $0{,}2 > P > 0{,}1$ |
| » » | Inferior ..... | 65,27 ± 5,85 % | | 15 | |
| Total | Equivalent or better | 79,50 ± 3,43 % | 2,48 | 20 | $0{,}02 > P > 0{,}01$ |
| » | Inferior ..... | 65,77 ± 4,09 % | | 26 | |

According to this method of computation the difference within the morphological group is rather considerable, but is less prominent within the physiological group.

Of the eleven mutants in the morphologically drastic group, which in general show a low $X_1$ fertility, only two have so far been tested in yield trials. They were conspicuously inferior. The rest set little seed. If they had been tested in yield trials also, the correlation coefficient would undoubtedly have increased considerably.

If this view is correct, the plant-breeder who wishes to employ mutations in his work must seek the middle way between two extremes, (1) he ought to produce so great a number of vital mutants as possible, (2) he ought to exclude the lethalizing effect that appears after high

X-ray doses and high $X_1$ sterility. This difficulty was already stressed by STADLER in 1930.

## 4. CONCLUSIONS.

Among morphological mutants out of Golden barley, *erectoides* 1 and 7 were tested in yield trials over a long series of years, the former also at some of the branch stations of the Swedish Seed Association. In fifteen series of trials (each with several replicates) it gave a relative yield of 101. It shows a substantially increased straw-strength and may be denoted as a progressive variant.

Maja barley has the highest yield of all the sufficiently tested South-Swedish strains. In six series *erectoides* 12 gave a relative value of 100 and showed a considerably increased straw-strength. *Erectoides* 16, with better straw-strength and a considerably higher 1000-grain weight, gave — in spite of its increased earliness by six or seven days — a relative value of 95 in six trial-series. Both these mutants may be denoted as progressive. The same applies to *erectoides* 13 with its enormous straw-strength.

Among the physiological mutants out of Golden barley special mention may be made of a »late and tall mutant», (44/2), which was studied in eleven trial-series and gave the relative value of 104. The high yield is probably associated with the longer and kernel-richer ears. Its ecological requirements seem to be changed. The straw-strength is about the same. A very waxy, peculiar mutant (44/3) was awarded the relative value of 99 in twelve series, and belongs to the straw-stiffest observed in the Scandinavian assortment. Unfortunately, its 1000-grain weight is very low. Mutant (44/7) is extremely high-yielding, with an exceedingly high 1000-grain weight (more than 10 % above that of Golden barley). In six trial series it gave the relative figure 109, thus ranking almost level with the Maja barley.

Of still greater interest, of course, are the best physiological mutants out of Maja barley. In five series the mutant 44/18 gave the relative value of 99, but, moreover, is considerably straw-stiffer and ripens quicker. The best, however, seem to be the mutants 44/89 and 44/76, which, to be sure, were only tested in the year 1945 but in several pedigree series were then about 10 % above Maja barley. In addition, 44/89 has a more copious tillering, a higher kernel number per ear, and ripens earlier than the mother-line.

The highest yielding mutants were also investigated with respect to their malting properties. In some, the diastatic activity ($\beta$-amylases) and starch liquefying power ($\alpha$-amylases) had been considerably reduced. In Golden barley with its relatively low content of decomposing enzymes valuable variants seem able to arise comparatively easily. The most interesting is a mutant that reproduces perfectly the Golden-barley type in outer respects but deviates by somewhat broader leaves and an essentially increased 1000-grain weight. The last-mentioned quality is accompanied by a considerably improved malting quality. This is also the case in the large-kernelled mutant 44/7, which in addition produced a rich yield. The highest producing mutant from Maja (44/89) likewise seems to possess improved malting properties.

Finally, it is shown in this Chapter that a weak but distinct correlation exists between on one hand the $X_1$ fertility of the mutated original plants (a measure of the X-ray effect) and on the other hand the yield of the pure-bred mutant, in

conformity with STADLER's assumption (1930). A mutation with a positive action is often accompanied by mutations having lethal effects.

## VII. MUTANTS IN OTHER AGRICULTURAL PLANTS.

### 1. WHEAT.

*Earlier results.* — In the diploid species *T. monococcum* and *aegilopoides* (SMITH, 1936, 1939) spontaneous as well as induced chlorophyll aberrants are readily formed. These are rare in the tetraploid stage and almost never occur in the hexaploid. FRÖIER (1946) has especially stressed the different facility with which chlorophyll mutants arise in hexaploid oats and hexaploid wheat. The hexaploids of the two genera are probably genomically differently constructed.

In wheat polyploids also, however, a couple of cases of chlorophyll aberrants have been observed, especially after crosses between tetraploid species among themselves and between tetraploids and hexaploids (FRÖIER, l. c.). An undoubted chlorophyll mutation, spontaneously arisen, was observed by NEATBY (1933) in hexaploid wheat. It was of *virescens* type, i. e. white as seedling but turned green later and could even produce seed in the homozygous state. This *virescens* type proved to be instable, for it mutated (1) back to the normal state, (2) to pure *albina*, and (3) to a second *virescens* type with considerably slower chlorophyll formation. SCHWARNIKOW described (1936 a, p. 477) a pale-green normally fertile mutant, which may belong to the *viridis* group and which arose in the progeny of heat-treated seed. He likewise found solitary »chlorophyll mutations» in progenies from aged seed (1937).

An important group of lethal or sublethal mutants consists of the so-called speltoids, which were first described by NILSSON-EHLE (1917) and were later investigated by him and a long series of other workers. The speltoids may be divided into A, B and C types, according to disturbances found in the segregation and specific cytological anomalies. Of special value for the whole speltoid problem was WINGE's hypothesis (1924) of the connexion of these mutations with chromosome deviations. UCHIKAWA (1941) has recently published a survey of this region of investigation. The so-called compactoids arise as secondary phenomena in the $F_2$ and $F_3$. The frequency of the speltoid mutations ranges from about 0,1 to 1 %.

SCHWARNIKOW (1937, 1939) has shown that the number of speltoids as well as of sterility mutants, dwarfs, etc. is very substantially increased in progenies from aged seed. Seed that has been stored for about ten years germinates very badly: in twelve samples (9—11 years old seed) of two varieties the germination amounted to 40 %, while the control showed 88 %. In the same material the mutation frequency (calculated on the number of plant progenies) increased to 17 %, the majority of which consisted of lethal and sterile products, especially speltoids.

The same author also found (1936) that temperatures of 40°—60° C. considerably raise the number of mutants in the $I_2$ progenies. A temperature of 40° for 21—31 days produced an aberrant frequency of 32 %, 60° for 16—26 days a frequency of 45 %. The mutants that were isolated usually possessed a lethal character; they were dwarfs, narrow-leafed variants, speltoids, small-eared and light-green types, etc. But there also appeared vital mutants with increased waxiness, red-brown ears, rather dense ears, and increased straw-strength. The control material, which however was not particularly large, did not give a single aberrant. This method of inducing vital mutants requires further study. It can be definitely asserted that it

has not the same effect in diploid barley, even at higher temperatures. Still, the results are worthy of the greatest attention.

LEWICKI (1922) found in *T. durum* var. *coerulescens* a mutant with »fragile spikes» and hairiness at the base of the spikelets. These properties characterize *T. dicoccoides*, which is regarded as an ancestor of cultivated wheat. In addition, the mutant differs considerably from the parent line by its longer straw and spikelets.

COFFMAN (1924) observed solitary aberrants out of the variety Mindum from *T. durum*, probably arisen through mutation and characterized by supernumerary spikelets in the lower portion of the heads. Similar forms have been described by MEUNISSIER (1918), PERCIVAL (1921) and KAJANUS (1924), but it has not been clarified whether they have arisen by mutation or segregation.

CHRISTIANSEN-WENIGER (1926) found a peculiar mutant in the spring-wheat variety »Green Mountain» with a very wide range of modification, the form of the ear varying from long and spelta-like to dense compactoid, in extreme cases deviating greatly from the average type without there being hereditary differences.

KOLKUNOV (1928; Biol. Abstr. 7, No. 17723) measured year after year the stomatal length in a number of pure lines of wheat. Out of the strain »Banatka», itself rather large-celled, a form with exceptionally large stomata and reduced awns, probably arising by mutation, was isolated in extensive selection experiments. The mutant was to some extent dwarf-like, had a feeble reproductive system, and was sensitive to frost and drought as well as to attacks of saw flies (*Cephus* sp.). KOLKUNOV considers that »a mutation in cell-size may appear in any pure line».

DEKAPRELEVICH (1929), according to Biol. Abstr. 7, No. 343, has observed a mutant with a long lax spike, long rachillas, and coarse wide empty glumes, maturing more slowly than the parent line. He compares the mutant in question with the speltoid type and explains the strong pleiotropism as a result of a group of linked genes having mutated.

X-ray mutants have been produced in diploid species (*T. monococcum*, STADLER, 1929; SMITH, 1936, 1939), tetraploids (*T. durum*, SAPEHIN, 1935), hexaploids (DELAUNAY, 1930, 1931, 1932, 1934; SAPEHIN, 1930, 1934, 1936; FONDARD et CABASSON, 1939; RANJAN, 1940; GUSTAFSSON, 1941 a). Cf. FRÖIER, 1946 a. STADLER produced chlorophyll aberrants only in *T. monococcum* and found there a mutation frequency comparable with that of barley. In the same species as well as in the closely related *T. aegilopoides* SMITH isolated about 400 mutants, 80 of which, i. e. every fifth mutant, were viable in the homozygous form.

According to SMITH, solitary mutants are formed with a strong morphological effect accompanied by high vitality and fertility. To these belong mut. *biaristatum* (*ba-a*): well-developed awn on the lemma of each of the two florets of a spikelet; mut. *compactoides* (*c-1*, *c-2*): rachilla shortened, the mature plant somewhat dwarfish with thick, bristle culms; mut. »awned glumae» (*ga*): both outer glumes with awns, the awn of the lemma reduced, glumes soft, seeds easy-shelled; mut. »wiry» (*wi*): young leaves narrow and stiff, fertile only in green-house cultures. In addition there is the morphological mutant *densinodosum* (*mi*): many and short internodes. The mutant does not develop seed in field cultures, and does so poorly in the green-house. Of very great interest are two earliness mutants (*e-1* and *e-2*), one of which under field conditions is three weeks earlier than the mother-line. The other is not quite so extreme but is more vigorous.

The Russian investigators cited above, who worked with *T. durum* and *T. vulgare*, have attained valuable results. They have generally irradiated growing plants at anthesis. In this way extremely powerful chromosome disturbances are induced that are unmasked in the progeny from the irradiated plants. The $X_2$ plants are »extremely polymorphic» and in the great majority of cases »characterized by chromosome aberrations» (SAPEHIN, 1936, p. 36). (Cf. also DELAUNAY, 1930). A close positive correlation was found between the degree of nuclear abnormalities and the degree of phenotypic changes.

*T. vulgare* has been carefully studied. DELAUNAY (1934) divides the mutants produced into seven groups: (1) awned forms, (2) speltoids, (3) dense-eared, (4) point-glumed forms, (5) dwarfs, (6) other clear mutants, (7) undoubted mutants that are difficult to characterize. In certain cases an induced mutation frequency is obtained of 60 % as against a spontaneous frequency of 0,1 %, i. e. an increase by 600 times. SAPEHIN (1936) finds minor variations in the form of the glumes and in the density of the spikes, numerous speltoids and semispeltoids, single dwarfs, etc. Spring and winter forms behave similarly.

In SAPEHIN's experiments (1935) *T. durum* gave fewer mutants than *T. vulgare*. There would however seem to exist no fundamental difference in behaviour.

The two Russian investigators lay great stress on the value for plant breeding of the results obtained. »Artifical mutations are becoming a valuable method in plant breeding» (SAPEHIN, 1935). In his comprehensive work of 1934 DELAUNAY expresses himself to the same effect. Whether some of the induced mutants actually obtained practical value in the USSR, is not known. No yield figures have been published. The method of X-raying growing plants certainly gives many mutants but is disadvantageous from other points of view.

GUSTAFSSON (see 1941 b, p. 241) X-rayed in the mid-thirties seed from Pudel wheat (a white-kernelled variety) and obtained speltoids, straw-height mutants, dense-eared straw-stiff types, several of them fully vital to judge from appearances. They have however not been studied further. Pudel wheat is now devoid of any practical value. In this case it can be positively asserted that spontaneous crossing or contamination of seed cannot account for the origin of the variants.

MORITZ-VOM-BERG (1935) described a mutant in *T. vulgare* produced by treatment with acetic acid, and BYNOV (1938) considered that he had induced mutants by means of electric currents.

*New results.* — Experiments carried out jointly by FRÖIER and GUSTAFSSON with a view to producing practically valuable variants by the mutation method have confirmed the results of the Russian investigators. *With suitable X-ray doses a mass mutating sets in*, not only with regard to speltoids but also compactoids, awn mutants, vital mutants with changed straw-height, straw-strength, earliness, tillering. On the base of the pure line or the homozygotized cross-product there arises a new polymorphy that is not inferior to that of the original land varieties. The survey given below illustrates this (for further details, see FRÖIER, 1946 a, b):

| | Number of $X_2$ families | Number of progenies segregating for speltoid mutants | Other mutations |
|---|---|---|---|
| Spring wheat | | | |
| Kolben 10.000 *r* | 16 | 3 = 19 % | Ear density, length of awns |
| » 10.000 *r* | 207 | 89 = 43 % | Ear density, length of straw, breadth of leaves |
| » 15.000 *r* | 33 | 12 = 36 % | Length of straw, tillering, earliness |
| Diamond II 10.000 *r* | 100 | 46 = 46 % | Straw stiffness, length of awns, ear density, earliness |
| Winter wheat | | | |
| Gluten 20.000 *r* | 61 | 10 = 13 % | *Compactum*, straw height, ear density, awnness |
| Scandia III 15.000 *r* | 617 | 52 = 8 %[1] | *Compactum*, awnness, straw height, earliness, other properties |
| » » 20.000 *r* | 427 | 61 = 14 %[1] | *Compactum*, awnness, other properties |
| Controls | | | |
| Spring wheat | 100 | — | No obvious changes |
| Winter wheat | 339 | — | No obvious changes |

Thus, in certain cases the speltoid frequency amounts to 40—50 %. The number of other mutants is occasionally very high. In all, controls of 439 plant progenies have been examined, and in these not a single speltoid mutant has been observed with certainty. Scandia III is a commercial strain of high value, which is not yet completely uniform. Its variation was slight in the control tests as compared with that of the $X_2$ material.

Mr. J. MAC KEY, who has taken over the genetic and cytologic analysis of the numerous speltoid and compactoid cases, has made a further inventory of some of the $X_2$ material in Scandia III (20.000 *r*). In fifty $X_2$ families ten (20 %) were observed that contained speltoids, four (8 %) contained compactoids (partially modifications?), and thirty-one contained single or numerous long-eared types. Several of the last-mentioned are certainly distinguished from the normal type. Awn mutants and sterile forms have also been isolated.

[1] Preliminary calculation.

Although yield trials and quality analyses have not yet been carried out, it is clear that the wheat-breeder can produce vital mutants of practical value by means of X-ray treatment. The mutants appear in flocks to a larger extent than in barley, probably owing to the fact that the barley mutants are often derived from point mutations, while the wheat mutants are associated with chromosome aberrations, point mutations here only exceptionally giving a phenotypic effect. FRÖIER, GELIN and GUSTAFSSON (1941) found, after 20.000 *r*-units, no fewer than 94 % disturbed cell-divisions in the germinating seed of *T. vulgare*. Chlorophyll mutants of *albina* type have never been observed, although solitary ones of *viridis* or *alboviridis* type have. According to GUSTAFSSON, these are to a large extent due to chromosome disturbances.

## 2. OATS.

*Earlier results.* — Spontaneous chlorophyll mutants arise now and then also in the hexaploid *Avena sativa* (see Table 1 in FRÖIER, 1946 a). Both plastid and chromosome mutations are known, the latter usually being of *albina* or *viridis* type. Of special interest is ÅKERMAN's investigation of a sublethal *lutescens* variant (1922). The monoheterozygote can here be distinguished, while di- and tri-heterozygotes have a normal appearance. This chlorophyll aberrant, however, was not formed by mutation but after a cross between a monofactorial and a bifactorial line. The *chlorina* type described by ÅKERMAN and FRÖIER (1941) arose, on the other hand, as a mutation.

More or less sterile dwarfs arise not so infrequently (WARBURTON, 1919; STANTON, 1923; GOULDEN, 1926). STANTON observed both a recessive and a dominant dwarf. ZHEGALOV (1920) found a peculiar mutation that caused giant growth.

The so-called fatuoid mutants must be denoted if anything as sublethal. They were first described by NILSSON-EHLE (1911 a), who also showed that the heterozygote is intermediate and referred it in 1921 to the category of complex mutations. HUSKINS (1927, 1933) divided the fatuoids, in agreement with the speltoids in wheat, into different groups and considered them to have arisen as a consequence of chromosome aberrations. JONES (1930), who also isolated so-called subfatuoids, approached NILSSON-EHLE's view. The repeatedly advanced opinion that the fatuoids were cross-products between *A. sativa* and *fatua* has been definitely refuted (ÅKERMAN and BADER, 1938).

Far more important from the point of view of vitality are the kernel-colour mutants. These were also first described by NILSSON-EHLE (1911 b). In a seed-parcel of 700 kilograms of black oats he found 2.891 deviating kernels, 2.774 of which were grey, 115 white (and 2 yellow). Most of the grey and white kernels had probably arisen through mutation. ÅKERMAN and FRÖIER (1941) found one mutated kernel per 2.000 kernels in the widely cultivated black-oat varieties Engelbrekt II and Stormogul. The two commercial strains mentioned are monofactorial with regard to the black colour. By a successive transference of the cultivated varieties that are monofactorial to bifactorial, the number of white and grey kernels in the seed is

brought down substantially. The kernel-colour mutants are vital, although the yield is somewhat reduced (ÅKERMAN, 1929, p. 602).

A sporadic morphological mutant was described by COFFMAN and QUISENBERRY (1924). It arose out of Burt oats (*A. byzantina*) and showed very strong pleiotropism. The kernels were partially naked; 40—50 % of the caryopses were hulled by the threshing. In addition, the glumes were long, the spikelets 2—6-florate with elongated rachillas, causing the florets to extend beyond the glumes, and the ripening time shortened by several days.

In another case (COFFMAN, PARKER and QUISENBERRY, 1925) plants were observed in which the paleas of the kernels did not fuse with the caryopsis. In some of these plants the palea was curled away from the lemma, leaving the caryopsis exposed. The character was genetically controlled. Naked kernels were described before that by LOVE and MC ROSTIE (1919) for some plants in the variety »Sixty Day», but the nakedness was not transmitted here to the progeny.

According to MARTINET (1928), a peculiar variant, called »soldanelle», arose as a mutation out of a Swiss strain of oats. It is characterized by yellow kernels and is very early. The strain in question formed, in fact, »many mutations». The same variant also arose after a cross between the Swiss strain and a Canadian variety.

STADLER (1929) induced chlorophyll mutants in diploid *Avena* species, though not in *A. sativa*.

DERRICK and LOVE (1937) X-rayed a dwarf form of oats. From 332 caryopses there arose eleven fatuoids of dwarf type.

*New results.* — In his work of 1946, FRÖIER describes a large number of induced chlorophyll aberrants in diploid and hexaploid species. As yet, no such mutants have been obtained from tetraploids owing to insufficient material. Suitable doses for the three polyploid classes are 5.000, 5—10.000 and about 15.000 *r*. The chlorophyll mutants behave as in spontaneous material and can be grouped in the same way as was done for barley (p. 17). However, judging from the tables, *albina* mutants are relatively commoner in the diploid than the hexaploid representatives. This may be due to the fact that *albina* mutants arise to a large extent as gene mutations, while *xantha* and still more *viridis* types are largely correlated to gross chromosomal re-arrangements. Fifteen *albina* mutants in *A. strigosa* and *brevis* showed, as a matter of fact, an average fertility of 64 % in the $X_1$ plants from which they were derived, twelve *viridis* mutants a corresponding value of 41 %. The average difference thus amounts to 23 %.

Of great interest is the different mutability which FRÖIER found in hexaploid commercial strains after X-ray treatment.

| | | | | | | |
|---|---|---|---|---|---|---|
| Stormogul | gave | 12 | genetically | controlled | chlorophyll | aberrants |
| Victory<br>Golden Rain<br>Clock II | » | 1—3 | » | » | » | » |

| | | | | | | |
|---|---|---|---|---|---|---|
| Stormogul | gave | 12 | genetically | controlled | chlorophyll | aberrants |
| Abeds Nova<br>Sapeli<br>Engelbrekt II | » | 0 | » | » | » | » |

This difference seems to be due to unequal reduplication of chlorophyll-determining chromosome parts.

Induced fatuoids have not arisen at all in the Svalöf experiments. Nor has the frequency of the spontaneously so numerous kernel-colour mutants been noticeably increased.

*Vital mutants* have been obtained in *A. strigosa*, *A. brevis* and *A. sativa*. *A. strigosa* is cultivated very little in Europe, and then in dry and poor soils in northwestern Germany as well as in certain parts of Great Britain and Ireland, and hence it has but little economic value. Several mutants were isolated out of a line of this species, among others a form with a silvery colour on lemma and palea and a lower culm (mut. *argentea*, according to FRÖIER) and a substantially earlier, more rapidly withering form, likewise with a shorter culm. Further, physiological mutants that merely altered the straw-height (in a positive or negative direction) or tillered to different extents were not uncommon (FRÖIER, 1946 b).

In *A. brevis* there were observed mutants in straw-height, ripening time (a striking earliness mutant) as well as a mutant with the phylogenetically important property that the spikelets (the fruits) loosened on ripening.

In *A. sativa* mutants have been more especially obtained from the black-oat variety Clock II. This arose at the beginning of this century as a spontaneous cross between Golden Rain (white-kernelled) and Clock (black-kernelled). The length of the culm is rather easily influenced, positively as well as negatively. Awn mutants have been isolated. Different earliness mutants have been observed, one of which in comparative cultivation was specially striking. Besides the last-mentioned mutants, which have practical value, types have also arisen that ripen very late, up to fourteen days after the original line. Different gradations in lateness occur. In Stormogul, a black-oat line already isolated in 1889, solitary straw-height mutants have been induced. But this as well as the white-kernelled Victory oat, which has given a couple of lateness mutants, are more stable than Clock II with regard to vital aberrants (cf. Golden barley, Maja barley, Ymer barley, p. 49).

The results in hexaploid oat are very promising for future mutation work. FRÖIER has carried out a large number of crosses with the best earliness mutants.

### 3. FLAX.

During several years irradiation experiments have been carried out on fibre and oil flax (GRANHALL). The suitable X-ray doses are between 30.000—40.000 *r*. In the $X_2$ and $X_3$ generations strong selection has been carried out for tall and for short straw, high and low seed-content. Such a selection leads to tangible results. It has however been found that the spontaneous crossing frequency is rather high, and that pure lines grown next to one another gradually become heterogeneous as a consequence of hybridization. The results of the X-raying were therefore not unexceptionable, and the experiments had to be commenced afresh. No cases of chlorophyll mutations have been observed.

Such have however been demonstrated by LEVAN (1944). In the progeny from irradiated seed of diploid Hercules and Concurrent three mutants arose, two of which were lethal or sublethal. Cultivation of the third mutant (out of Concurrent) on a large scale has given extremely interesting results. In colour this mutant is yellowish-green and grows more slowly than normal plants; eventually, however, it attains the same height and sets abundant seed.

GRANHALL (1946, Table 1, p. 296) has found that the mutant is fully vital, in spite of the fact that it must be denoted as a chlorophyll aberrant (cf. mut. 42/325 in barley, p. 68). Its yield, so far as straw production is concerned, is above that of the mother-line. The seed-yield is about the same. At the same time, however, the content of long fibre is higher.

| | Straw yield kg/hectare | | | Seed yield kg/hectare | | | 1000-grain weight |
|---|---|---|---|---|---|---|---|
| | 1944 | 1945 | Relative yield | 1944 | 1945 | Relative yield | Average gr. |
| Concurrent .... | 5330 | 4570 | 100 | 1680 | 1350 | 100 | 5,6 |
| Mutant .... ... | 5630 | 4850 | 105,9 | 1750 | 1290 | 99,9 | 5,3 |

The mutant has a somewhat lower 1000-grain weight. The unaltered seed-crop ought therefore to be due to a higher number of capsules per plant or more seeds in each capsule.

Flax is of very great interest from a mutation point of view. At about the same time as BAUR, TINE TAMMES (1925) framed her hypothesis on the evolutionary importance of »Kleinmutationen». In the light of the rather high frequency of spontaneous crossing it is possible that many of the variants that she studied depend on segregation rather than on mutation.

### 4. SOME OTHER KINDS OF PLANTS.

A few words may be awarded to mutants out of soy-beans (ANDERSSON, 1944, p. 291). On account of the poor weather conditions in the years 1940—1942 a large part of the original material was ruined. Still, several interesting mutants have been obtained. Some may be denoted as morphological, for instance, those with another seed-colour, presence of anthocyanin, entirely new mode of growth. Physiological mutants have also arisen, though none unfortunately show so advanced an earliness as the corresponding types in barley and oats. The work is going on to a large extent. Two X-ray selections out of Altonagaard AI gave in the 1944 and 1945 trials the relative figures 116 and 108 as regards yield, and ripened three and two days earlier.

Extensive trials have been carried out on oil-turnips and mustard. These are not entirely self-fertilizing. From the common spring variety Regina oil turnips a homogeneous line has been obtained which ripens some days earlier and grows much faster in seedling stage. This is one reason it is not so severely attacked by flea-beetles. This new variety has been tested in a great number of yield trials in southern Sweden. In Svalöf its relative yield lies at 105 (mean of 1944 and 1945). In five other places its yield averages 111. Its content of fats is 39,3 % against 38,6 % for the original strain. *No similar variety has ever arisen in pedigree cultures.* Its striking habit of growth makes an origin by means of mutation highly probable.

In mustard several mutations have been isolated, some of them influence flower colour (different gradations from yellowish to white). Some X-ray pedigrees have increased yield considerably (44/113: rel. yield 113, 44/156: 109). A great number of lines have been tested. Their origin by means of mutations cannot be definitely stated. From a practical point of view this is of course immaterial.

Full accounts will be published by Dr. ANDERSSON.

Some interesting mutants have been isolated also in sweet-lupine. Data concerning these will be published later on by Dr. TEDIN.

## VIII. GENERAL SURVEY OF THE RESULTS.

*Mutation types.* — In cereals spontaneous and induced mutants may be conveniently divided into three groups: (1) chlorophyll mutants, (2) sterility and lethality mutants of different kinds, and (3) vital mutants. The chlorophyll mutants are so characteristic that they should strictly be kept separate from the sterility and lethality mutants. Spontaneous and induced chlorophyll mutants are common in diploid organisms but become rare in the polyploid. This applies especially to *albina* types. In polyploid wheat and oat species speltoid and fatuoid mutants take the ascendancy instead.

The vital mutants are characterized by normal fertility and show no noteworthy lethality in the vegetative stage. They may conveniently be divided into a morphological and a physiological subgroup. The yield is usually more or less reduced. In barley the so-called erectoid mutants are especially striking.

*Mutation frequencies.* — From the extensive series of experiments laid down in diploid barley it seems evident that pure lines have a very high stability. On the other hand, the mutability rises considerably in cross-strains, even in such as are homozygous judged from external signs. It is possible that minor structural aberrations still occur and that these cause irregularities in meiotic pairing. In this way new structural aberrants may be formed, and some of them have a phenotypic effect. It is also possible that the genes are labile owing to the recombination itself, i.e. an unselectioned genotypical environment.

Even in very stable genotypes the mutation frequency rises considerably if the external conditions are altered, e.g., by heat treatment or old age of the seeds. In the analysed cases the effect corresponds to an X-ray dose of 500 *r*, and is thus rather strong.

One induced morphological mutant in barley — fertile or infertile — corresponds in frequency of occurrence to 22 chlorophyll aberrants, 15 mutants that prevent normal meiosis and gamete formation, and about 30 cases of »translocation sterility». In order to obtain one mutant with a distinctly higher yield the barley-breeder must induce about 700—800 worthless types at the same time.

As a contrast to these figures it may be mentioned that of three mutants in flax which arose in Dr. LEVAN's experiments one proved to be clearly superior to its mother-line. In this case luck and material favoured the mutation investigator.

*Practically valuable mutants.* — The primary object of the investigations described above has been to determine to what extent in-

Fig. 12. Two ears of an extreme mutant in barley, showing long ear internodes and a greatly increased number of spikelets in the fertile rows. Isolated in 1946. Different gradations occur. Mother strain: Ymer 40/13 $b_7$.

duced and spontaneous mutants meet the plant-breeder's demand for cultivating value. In the best cases the yield ought to have risen, the straw-strength increased, the 1000-grain weight highered, the quality

improved. It is however to be regarded as a gain if any one of these can be attained. The plant-breeder then has it in his power to transfer solitary good qualities from otherwise poor mutants to already existing commercial varieties. STADLER was in 1930 rather sceptical as to the possibility of improving cereals by means of induced mutations. The variation at the disposal of the plant-breeder would still suffice, he thought, a good bit forward. That may be so, but in the same year ELISABETH SCHIEMANN pointed out in an overlooked paper that the good straw-strength in a widely spread German malting-barley strain was derived from a mutant inferior in most qualities.

Certain mutants yield more than their mother-line. The yield is undoubtedly a measure of the general vitality, both as regards the vegetative and reproductive phase. Thus, high yield points to increased vitality (under the prevailing conditions of cultivation). The results from the two barley strains most carefully examined here supplement each other. One strain, Golden barley, is an old, pure line, with the same qualities now as when it was isolated. It is manifestly very stable; mutations and spontaneous crossings are rare. Maja barley, the other strain, is not an original pure line in the same sense as Golden barley but a cross-product, which has become gradually homozygotized. Up till now its high yield has scarcely been surpassed by any other varieties of Scandinavian two-rowed barley.

With reference first of all to *the morphological mutants*, some of these attain to the yield of the maternal lines or can even surpass them. This applies especially to certain bright-green mutants, though also to such sharply defined types as *erectoides* 1 and 12. In the two last-mentioned the straw-strength has also risen. Individual properties such as 1000-grain weight and earliness may also change in a favourable direction. Especially remarkable is *erectoides* 16, which ripens one week before the mother-line and has only a slightly inferior yield, just as in fact *erectoides* 13, which is immensely straw-stiff. Mutants can thus be formed which deviate very much while retaining the productive power of the original line. The fully fertile morphological mutants give on an average a lower yield than the corresponding physiological ones.

Out of Golden barley two or three *physiological mutants* have been induced which are superior in yield to the mother-line. One of them (44/7) is 9 % higher. This valuable quality is probably connected with the greatly increased grain weight. Another mutant, 44/2, ripens a little later and is considerably richer in straw. Its yield varies greatly,

depending on the amount of rainfall during the vegetation period. The Svalöf average for six years, however, lies at a relative value of 103.

Still more interesting, of course, are the yield-raising mutants out of Maja barley. Three different pedigree lines from mut. 44/89 have given on an average 10 % more than the mother-line. They resemble the latter in most respects. High-yielding mutants are also 44/76, 44/91 and 44/30, the last-mentioned of which has been tested in rather extensive trials. As the Scandinavian plant-breeders have not succeeded, despite fifteen years of continuous work, in forcing the yield of the barley noticeably above the level of the Maja strain — either by selection or crossing — the best mutants must be assigned very great theoretical and practical interest.

Methodical work must therefore sooner or later lead to a raising of the yield by the mutation method as well, even in the case of the chief commercial varieties. It must not be considered as fundamentally impossible that an induced or spontaneous cereal mutant becomes so worth cultivating that it can immediately replace the original strain. The high requirements exacted by the plant-breeder of every new market variety will presumably make such a result less probable; impossible it is not. (Cf., for instance, the successful variant obtained in oil turnips, which will possibly be brought into the market in 1947.)

The old land varieties were exceedingly heterogeneous, but the value of the strains contained in them varied. On the basis of the commercial strains it is now possible to produce a new polymorphy by means of mutation. The average yield of the tested *fully fertile and vital* physiological mutants lies only about 5—10 % below that of the original line. At the same time, however, the original line has been broken up in many different directions. The straw-strength has sometimes been improved, sometimes deteriorated, sometimes it is *status quo*. The same applies to earliness, 1000-grain weight, malting properties. Such mutants have been described in great numbers. For brewing purposes a mutant out of Golden barley is of special interest. Externally it is a faithful copy of the maternal line, in a mixed crop with this it would not be distinguishable. The yield is exactly the same. The leaves, however, are somewhat broader and the 1000-grain weight higher. In addition, it must be denoted as an entirely new and improved malting variety (THUNÆUS, 1946).

A mutant, suspected already in 1945 and certified in 1946, falls outside the known variation sphere of Scandinavian two-rowed barley with regard to floret and kernel number of the spikes. In the com-

mercial strains the fertile spikelets attain an average number of 24—28 (cf. the similar statement made by ÅBERG and WIEBE, 1946, p. 14, for American varieties). Several progenies of this mutant show a spikelet number up to 36—38, in other instances up to 34—36, i.e. an increase with 30—40 %. *If* this property can be combined with a higher straw-stiffness (the spikes are very heavy), and possibly some other valuable properties, the mutant may entirely renew Scandinavian barley breeding. It arose from the high-bred strain Ymer $b_7$.

Another mutant, *erectoides* 13, seems to have improved the straw-stiffness above the level of commercial barley strains. *Erectoides* 16 most certainly breaks the frame with regard to earliness. Both yield also well. Other mutants have been found that raise the kernel weight considerably and simultaneously improve yield. These examples, better than long statements, illustrate the possibilities involved in methodical mutation experiments.

An interesting case that visualizes the interaction between mutation, yield and environment has been observed in a chlorophyll mutant out of Golden barley. A golden-bright vital aberrant produced at Svalöf yielded 10 % below the mother-line. Cultivated about 1000 kilometres further north, it yielded 25 % above Golden barley in 1943 and 1944 in preliminary experiments. Probably in this case length-of-day conditions played their part, favouring the assimilation. Similar conclusions have been drawn by GRANHALL and LEVAN for a yellowish-green chlorophyll mutant in flax, induced in the foreign strain Concurrent. The seed production is the same, but the straw crop per hectare has increased by 6 % and also the fibre quality has been improved. This remarkable result shows that »defect or loss mutations» may imply direct improvements.

*Morphological effect and vitality.* — According to the results obtained here, the effect of the mutations and the yield of the mutants are intimately connected. This is excellently illustrated by the erectoid mutants. The highest productive types deviate only in a few characters from the maternal line and differ slightly in general habit. When straw-height and ear-length have changed considerably in one or the other direction, or new kernel characters have been induced, the production also falls. The same condition applies to the mutual yield of the three groups of bright-green, erectoid and drastic mutants (GUSTAFSSON, 1946 a). The relative figures average 101, 88 and 84 for the three groups. Moreover, certain drastic mutants are entirely sterile.

*Lethal factors and yield.* — STADLER advanced the assumption in 1930 that solitary good mutations can be produced by X-ray treatment but that at the same time lethal factors are induced in great number. This assumption has been confirmed, although the correlation between the yield of the mutants and the sterility of the original plants (a measure of the lethalization) is not especially strong. Nevertheless, this correlation is rather clear for the mutants out of Golden barley. Most seed-material of this line has been subjected to high X-ray doses and special pre-treatment. By this means a considerable $X_1$ sterility has been induced. The correlation is weaker in mutants out of Maja barley, where the X-ray effects have been less violent. One case that excellently illustrates STADLER's assumption may be mentioned. Among others, a peculiarly waxy, extremely straw-stiff mutant was isolated out of Golden barley. Its yield — calculated on all trials — is equal to that of the maternal line. The 1000-grain weight is low, however, and the malting properties have deteriorated. Thus, good and bad properties accompany each other. With SCHIEMANN's report in mind, however, the enormous straw-strength may no doubt be assigned some value in future crossing work.

*Control of the mutation process.* — Series of special experiments seem to show that the mutation process can be experimentally controlled. If the cell environment is altered in different ways, or the irradiation effect is varied, this causes quite different mutations and mutant types to arise. For instance, it may be regarded as certain, firstly, that, as opposed to *albinas, xantha* mutants are not produced in the progeny of entirely fertile $X_1$ plants, secondly, that *alboxantha* types preferably arise from irradiated germinating seeds or seeds rich in water, thirdly, that for their origin the so-called drastic mutants require a very strong irradiation effect upon the treated seeds.

The results obtained so far at Svalöf and abroad are certainly rather meagre. Continually extensive experiments are being carried out, however, with the express purpose of elucidating these questions from a practical point of view.

In conclusion stress must once more be laid on what has here been adduced respecting the yield of the vital mutants, viz. that the results in diploid barley seem to apply to all agricultural plants so far examined, whether they are diploid or polyploid. A methodical work will sooner or later lead to positive results. The plant-breeder cannot

neglect the artificially induced mutants for the further improvement of his varieties.

Svalöf, June 1st, 1946.

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## LITERATURE CITED.


1. AASE, H. C. 1946. Cytology of cereals. II. — Bot. Rev. 12: 235—334.
2. ANDERSSON, G. 1944. Redogörelse för arbetena med soja vid Sveriges Utsädesförening åren 1941—1943. — Sveriges Utsädesf. Tidskr. 54: 279—293.
3. BRESLAVETZ, L. P. *et al.* 1936. Increasing yield in rye by X-ray irradiation. — Kosmos, Lwov, 8: 288. (Cited after AASE, 1946.)
4. BURGEFF, H. 1943. Genetische Studien an *Marchantia.* — Jena. 296 pp.
5. BURNHAM, CH. R. 1946. A gene for »long» chromosomes in barley. — Records Gen. Soc. America 14. (Abstract.)
6. BYNOV, F. A. 1938. Les mutations de *Triticum vulgare* provoquées par un courant électrique. — Trad. Jard. Bot. Univ. Moscou 2: 34—35. (Cited after Res. Gen. 1940.)
7. CHRISTIANSEN-WENIGER, F. 1936. Über die Modifizierbarkeit der Form der Weizenähre durch die Jahreswitterung und erster Bericht über eine Variabilis-Mutation bei Weizen. — Zschr. f. Pflanzenzücht. 11: 313—339.
8. COFFMAN, F. A. 1924. Supernumerary spikelets in Mindum wheat. — Journ of Hered. 15: 186—192.
9. COFFMAN, F. A., PARKER, J. H. and QUISENBERRY, K. S. 1925. A study of variability in the Burt oat. — Journ. Agr. Res. 30: 1—64.
10. COFFMAN, F. A. and QUISENBERRY, K. S. 1923. A multiflorous variation in Burt oats. — Journ. of Hered. 14: 185—192.
11. DEKAPRELEVICH, L. L. 1929. A case of mutation in a pure line of wheat. — Tiflis Bot. Garden 6: 197—200.
12. DELAUNAY, L. N. 1930. Die Chromosomen-Aberranten in der Nachkommenschaft von röntgenisierten Ähren einer reinen Linie von *Triticum vulgare albidum* ALL. — Zschr. f. ind. Abst.- u. Vererb.-lehre 55: 352—355.
13. — 1931. Resultate eines dreijährigen Röntgenversuches mit Weizen. — Der Züchter 3: 129—137.
14. — 1932. The X-mutations in wheat. — Bull. Lab. Genet. Leningrad 9: 173—180.
15. — 1934. Experimentell erzeugte Mutationen bei Weizen. — Verlag Ukr. Akad. f. landwirt. Wiss. Pp. 1—56. (Cited after an abstract by SCHTERBINA in Res. Gen. 10: 164—165.)
16. DEMEREC, M. 1937. A mutability stimulating factor in the Florida stock of *Drosophila melanogaster.* — Genetics 22: 190.
17. DERRICK, R. A. and LOVE, R. M. 1937. Artificially induced fatuoids in a dwarf mutant oat. — Sci. Agric. 17: 703—706.
18. DICLUS, D. J. 1936. Beiträge zum Studium der spontanen Variabilität »reiner» Linien von *Hordeum.* I. Eine neue Varietät zweireihiger Kulturgerste *Hordeum distichum* var. *triaristatum* mihi. — Bot. Z. 21: 189—194. (Cited after Der Züchter 9, 1937, p. 102.)

19. DOTTERWEICH, H. und SCHMIDTKE, L. 1941. Auslösung spezifischer Mutationen bei *Drosophila melanogaster* nach chemischer Dauerbeeinflussung. I und II. — Zschr. f. ind. Abst.- u. Vererb.-lehre 79: 220—239.

20. EKSTRAND, H. 1932. Ein Fall von erblicher Asyndese bei *Hordeum*. — Sv. Bot. Tidskr. 26: 292—302.

21. EULER, H. v. und HEVESY, G. v. 1944. Wirkung der Röntgenstrahlen auf den Umsatz der Nukleinsäure im Jensen-Sarkom. II. — Arkiv Kemi, Mineralogi, Geologi (Stockholm) 17 A. No. 30: 1—60. — Also the same journal 1945, vol. 19 A. No. 13 (together with L. AHLSTRÖM): 1—16.

22. FONDARD et CABASSON. 1939. Influence des rayons X et des ondes courtes. — C. R. Hebd. des Séances de l'Acad. d'Agric. de France 25, No. 13: 506—509.

23. FREISLEBEN, R. und LEIN, A. 1942. Über die Auffindung einer mehltauresistenten Mutante nach Röntgenbestrahlung einer anfälligen reinen Linie von Sommergerste. — Naturw. 30.

24. — und —. 1943 a. Vorarbeiten zur züchterischen Auswertung röntgeninduzierter Mutationen. I. Die in der Behandlungsgeneration ($X_1$) sichtbare Wirkung der Bestrahlung ruhender Gerstenkörner. — Zschr. f. Pflanzenzücht. 25: 235—254.

25. — und—. 1943 b. Vorarbeiten zur züchterischen Auswertung röntgeninduzierter Mutationen. II. Mutationen des Chlorophyllapparates als Testmutationen für die mutationsauslösende Wirkung der Bestrahlung bei Gerste. — Zschr. f. Pflanzenzücht. 25: 255—283.

26. FRÖIER, K. 1941. Keimung und Triebkraft bei Hafer und Weizen nach verschiedenen Röntgendosen. — Hereditas XXVII: 360—370.

27. — 1946 a. Genetical studies on the chlorophyll apparatus in oats and wheat — Hereditas XXXII: 297—406.

27 a. — 1946 b. Nya erfarenheter rörande röntgenmutationer hos vete och havre samt deras betydelse för kombinationsförädlingen. — Lantbruksveckan 1946: 225—242.

28. FRÖIER, K., GELIN, O. and GUSTAFSSON, Å. 1941. The cytological response of polyploidy to X-ray dosage. — Bot. Not. (Lund): 199—216.

29. FRÖIER, K. and GUSTAFSSON, Å. 1941. The influence of X-ray dosage on germination and sprouting ability in barley and wheat. — Sv. Bot. Tidskr. 35: 43—56.

30. — and —. 1944. The influence of seed size and hulls on X-ray susceptibility in cereals. — Hereditas XXX: 583—589.

31. GELIN, O. 1941. The cytological effect of different seed-treatments in X-rayed barley. — Hereditas XXVII: 209—219.

32. GENTER, C. F. and BROWN, H. M. 1941. X-ray studies on *Phaseolus vulgaris*. — Journ. of Hered. 32: 39—44.

33. GERSHENSON, S. 1940. Types of mutations induced in *Drosophila* by the sodium salt of thymo-nucleic acid. — Compt. Rend. Acad. Sci. URSS 26: 601—603.

34. GOODSPEED, T. H. 1929. Cytological and other features of variant plants produced from X-rayed sex cells of *Nicotiana tabacum*. — Bot. Gaz. 87: 563—582.

35. GOODSPEED, T. H. and AVERY, P. 1934. The cytogenetics of fourteen types

derived from a single X-rayed sex cell of *Nicotiana tabacum*. — Journ. of Gen. 29: 327—353.

36. GOULDEN, C. H. 1926. A genetic and cytological study of dwarfing in wheat and oats. — Minn. Exp. Sta. Techn. Bull. 33: 3—37.
37. GRANHALL, I. 1944. Svalöfs Ymerkorn (Sv 40/13 $b_1$). — Sveriges Utsädesförenings Tidskrift 54: 237—239.
37 a. — 1946. Lin och hampa. — Sveriges Utsädesf. Tidskr. 56: 290—299.
38. GUSTAFSSON, Å. 1937. The different stability of chromosomes and the nature of mitosis. — Hereditas XXII: 281—335.
39. — 1938. Studies on the genetic basis of chlorophyll formation and the mechanism of induced mutating. — Hereditas XXIV: 33—93.
40. — 1940. The mutation system of the chlorophyll apparatus. — Lunds Univ. Årsskr. N. F. Avd. 2. Bd. 36. Pp. 1—40.
41. — 1941 a. Mutation experiments in barley. — Hereditas XXVII: 225—242.
42. — 1941 b. Preliminary yield experiments with ten induced mutations in barley. — Hereditas XXVII: 337—359.
43. — 1942 a. Mutationsforschung und Züchtung. — Der Züchter 14: 57—64.
44. — 1942 b. The plastid development in various types of chlorophyll mutations. — Hereditas XXVIII: 463—492.
45. — 1944. The X-ray resistance of dormant seeds in some agricultural plants. — Hereditas XXX: 165—178.
46. — 1946 a. Drastic morphological mutations in barley. — Hereditas XXXII: 120—122.
47. — 1946 b. The origin of *albina* and *xantha* mutations in barley. — Acta Radiologica 27: 300—307.
48. GUSTAFSSON, Å. and ÅBERG, E. 1940. Two extreme X-ray mutations of morphological interest. — Hereditas XXVI: 257—261.
49. HALLQVIST, C. 1923. Gametenelimination bei der Spaltung einer zwerghaften und chlorophylldefekten Gerstensippe. — Hereditas IV: 191—205.
50. — 1924. Chlorophyllmutanten bei Gerste. — Hereditas V: 49—83.
51. — 1926. Koppelungen und synthetische Letalität bei den Chlorophyllfaktoren der Gerste. — Hereditas VIII: 229—254.
52. HARLAN, H. V. 1931. The origin of hooded barleys. — Journ. of Hered. 22: 264—272.
53. HARLAN, H. V. and POPE, M. N. 1922. Many-noded dwarf barley. — Journ. of Hered. 13: 269—273.
54. HONECKER. 1936. Die Bedeutung von Letal- und Defektfaktoren bei der Gerste. — Forschungsdienst, Sonderheft 3: 80—92.
55. HOR, K. S. 1922. A new variety of barley with striking characteristics. — Science 55: 378.
56. HRUBY, K. 1935. Durch X-Strahlen hervorgerufene abnorme Plastiden. — Z. Vest. Král. Ces. Spol. Nauk. Tr. II. Roc. 1935 (Praha): 1—5 (reprint).
57. HUBER, J. A. 1932. Eine atavistische Variation bei der Gerste. — Der Züchter 4: 33—35.
58. HUSKINS, C. L. 1927. On the genetics and cytology of fatuoid or false wild oats. — Journ. of Gen. 18: 315—364.
59. — 1933. The origin and significance of fatuoids, speltoids and other aberrant forms of oats and wheat. — Proc. World's Grain Exhib. Conf. Can. 2.

60. IKENO, S. 1925. Studien über die mutative Entstehung eines »*intermedium*»-Typus bei Gerste. — Zschr. f. ind. Abst.- u. Vererb.-lehre 37: 210—228.

61. IMAI, Y. 1935. Non-viable leek-like mutant seedlings of barley. — Jap. Journ. of Gen. 11: 282—283.

62. ISENBECK, K. und HOFFMANN, W. 1942. Gerste, *Hordeum sativum* JESS. — Handb. d. Pflanzenzücht. Bd. II: 130—224.

63. JONES, E. T. 1930. Morphological and genetical studies of fatuoid and other aberrant grain-types in *Avena*. — Journ. of Gen. 23: 2—67.

64. KAGAWA, F. 1939. Alteration of characters in crop plants induced by X-ray irradiation. — Jap. Journ. of Bot. 10: 35—41.

65. KAJANUS, B. 1924. Über eine eigenartige Ährenanomalie bei Weizen. — Hereditas V: 217—221.

66. KAPLAN, R. 1940. Experimentelle und theoretische Untersuchungen über den Mechanismus der Keimungsschädigung durch Röntgenstrahlen beim Pollen von *Antirrhinum majus* L. — Biol. Zbl. 60: 298—318.

67. KIESSLING, L. 1912. Über eine Mutation in einer reinen Linie von *Hordeum distichum* L. — Zschr. f. ind. Abst.- u. Vererb.-lehre 8: 48—78.

68. KNAPP, E. und KAPLAN, R. 1942. Beeinflussung der Mutationsauslösung und anderer Wirkungen der Röntgenstrahlen bei *Antirrhinum majus* durch Veränderung des Quellungszustandes der zu bestrahlenden Samen. — Zschr. f. ind. Abst.- u. Vererb.-lehre 80: 501—550.

69. KOLKUNOV, V. V. 1928. Inheritance of cell size. — Contr. Plant Breed. Inst. Kiev 4: 17—33.

70. KRAJEVOJ, S. J. 1937. Über die experimentelle Erzeugung von Mutationen bei Pflanzen. — Bull. Acad. Sci. URSS, Ser. Biol. No. 2: 427—458. (Cited after Der Züchter 10, 1938, p. 221.)

71. — 1939. On natural selection in populations. — Compt. Rend. Acad. Sci. URSS 24: 716—719.

72. — 1940. Experimental production of awned varieties of barley from awnless varieties. — Compt. Rend. Acad. Sci. URSS 29: 419—421.

73. LEA, D. E. 1946. Actions of radiations on living cells. — Cambridge Univ. Press. 402 pp.

74. LEVAN, A. 1944. Experimentally induced chlorophyll mutants in flax. — Hereditas XXX: 225—230.

75. LEWICKI, S. 1922. A new mutation in *Triticum durum* and its importance for the phylogenetics of wheat. — Mem. Inst. Nat. Pol. à Pulawy 3: 61—83.

76. LINDSTROM, E. W. 1933. Hereditary radium-induced variations in the tomato. — Journ. of Hered. 24: 129—137.

77. LOVE, H. H. and MC ROSTIE, G. P. 1919. The inheritance of hull-lessness in oat hybrids. — Amer. Nat. 13: 5—32.

78. LUTKOV, A. N. 1937 a. Experimentelle Gewinnung einer ligulalosen Form der Gerste unter dem Einfluss von X-Strahlen. — Trudy prikl. Bot. i pr. II Contrib. from the Labor. of Genet. of the Inst. of Plant Industry, No. 7. (Cited after Der Züchter 10, 1938, p. 108.)

79. — 1937 b. Die Erzeugung einer Winterform von *Hordeum* nach Röntgenbestrahlung. — The same journal and issue, cited after Der Züchter 10, 1938, p. 195.

80. LUTKOV, A. N. 1937 c. Durch Röntgenbestrahlung entstandene Chlorophyll-mutationen und andere Formen erblicher Variationen bei *Hordeum*. — The same journal and issue, cited after Der Züchter 10, 1938, p. 318.
81. MAC ARTHUR, J. W. 1934. X-ray mutations in the tomato. — Journ. of Hered. 25: 75—78.
82. MARTINET, G. 1928. Soldanelle, une même sort d'avoine très precoce, obtenue par hybridation et par mutation. — Verh. Schweiz. Naturf. Ges. 1928: 185.
83. MARTINI, M. L. and HARLAN, H. V. 1942. Barley freaks. — Journ. of Hered. 33: 338—343.
84. MEUNISSIER, A. 1918. Expériences génétiques faites à Verrières. Blé. VII. Epillets surnuméraires. — Bull. Soc. Nat. Acclim. France 65: 84.
85. MIÈGE, E. 1927. Apparition brusque d'une orge à barbes lisses. — C. R. Acad. Sc. Paris 184: 762—763.
86. MIYAZAMA, B. 1921. Dwarf forms in barley. — Journ. of Gen. 11: 205—208.
87. MORITZ-VOM-BERG, H. 1935. Zytologische Untersuchungen an der Nachkommenschaft künstlich erzeugter Weizenmutanten — Ber. deutsch. bot. Ges. 53: 548—559.
88. MÜNTZING, A. 1938. Sterility and chromosome pairing in intraspecific *Galeopsis* hybrids. — Hereditas XXIV: 117—188.
89. — 1941. Differential response to X-ray treatment of diploid and tetraploid barley. — Kungl. Fysiogr. Sällsk. Lund Förhandl. 11. No. 6: 1—10.
90. — 1942. Frequency of induced chlorophyll mutations in diploid and tetraploid barley. — Hereditas XXVIII: 217—221.
91. NEATBY, K. W. 1933. A chlorophyll mutation in wheat. — Journ. of Hered. 24: 159—162.
92. NILSSON-EHLE, H. 1909. Kreuzungsuntersuchungen an Hafer und Weizen. — Lunds Univ. Årsskr. N. F. 5, No. 2: 122 pp.
93. — 1911 a. Über Fälle spontanen Wegfallens eines Hemmungsfaktors beim Hafer. — Zschr. f. ind. Abst.- u. Vererb.-lehre 5: 1—37.
94. — 1911 b. Spontanes Wegfallen eines Farbenfaktors beim Hafer. — Verh. naturf. Ver. Brünn: 139—156.
95. — 1917. Untersuchungen über Speltoidmutationen beim Weizen. — Bot. Not. (Lund): 305—329.
96. — 1921. Über mutmassliche partielle Heterogamie bei den Speltoidmutationen des Weizens. — Hereditas II: 25—76.
97. — 1939. Polymere Röntgenmutationen bei *Hordeum*. — Nova Acta Leopoldina, Neue Folge, Bd. 6 (reprint).
98. PERCIVAL, J. 1921. The wheat plant. A monograph. — London. 463 pp.
99. PETO, F. H. 1937. Genetical studies on mutants in the progeny of heat-treated barley. — Canad. Journ. Res. 15: 217—229.
100. PISMENKO, P. A. 1937. Mutations in barley induced by X-rays. — Journ. Inst. Bot. Acad. Sc. URSS 1933, No. 13/14: 95—108.
101. POPE, M. N. 1935. Fifteen years of selection in six varieties of barley. — Journ. Amer. Soc. Agron. 27: 142—148.
102. RANJAN, S. 1940. A preliminary note on the X-ray mutants of Pusa (52) wheat. — Proc. Indian Acad. Sci. Sect. B, 12: 62—66.
103. RAPOPORT, I. A. 1940. Influence of thymonucleic and nucleic acids and of

some of their components on mutations. — Compt. Rend. Acad. Sci. URSS 27: 1033—1036.

104. RHOADES, M. M. 1938. Effect of the *Dt* gene on the mutability of the $a_1$ allele in maize. — Genetics 23: 377—397.

105. — 1945. On the genetic control of mutability in maize. — Proc. Nat. Acad. Sci. U. S. A. 31: 91—95.

106. SAPEHIN, A. A. 1930. Röntgenmutationen beim Weizen (*Triticum vulgare*). — Der Züchter 2: 257—259.

107. — 1934. Röntgenmutationen als ein Ursprung von Rassen der landwirtschaftlichen Pflanzen. — Priroda 9: 28—31. [Cited after Res. Gen. 11, p. (60).]

108. — 1935. X-ray mutants in *durum* wheat. — Bot. Z. URSS 20: 3—9.

109. — 1936 (1935). X-ray mutants in soft wheat. — Bull. Appl. Bot., Gen. a. Pl. Br. Ser. II: 9: 3—37.

110. SAX, K. 1939. The time factor in X-ray production of chromosome aberrations. — Proc. Nat. Acad. Sci. Wash. 25: 225—233.

111. SCHARNAGEL, TH. 1925. Über die Entstehung einer eigenartigen Abnormität als konstante neue Rasse bei Gerste. — Zschr. f. ind. Abst.- u. Vererb.-lehre 38: 134—139.

112. SCHIEMANN, E. 1930. Über eine praktisch und phylogenetisch wichtige Mutation bei Gerste nebst einigen Bemerkungen über Mutation bei Getreide. — Ber. Deutsch. Bot. Ges. 48: 477—489.

113. SCHWARNIKOW, P. K. 1936 a. Einfluss hoher Temperatur auf die Mutationsrate bei Weizen. — Planta 25: 471—480.

114. — 1936 b. Wirkung hoher Temperatur auf die Mutationsrate von *Crepis* bei verschiedener relativer Luftfeuchtigkeit. — Planta 25: 689—695.

115. — 1937. Über Erhöhung der Mutationsrate bei Weizen nach langer Aufbewahrung der Samen. — Genetica 19: 188—199.

116. — 1939. Mutation in seeds and its significance for a seedculture and selection of plants. — Bull. de l'Acad. Sci. URSS: 1009—1054.

117. SMITH, L. 1936. Cytogenetic studies in *Triticum monococcum* L. and *T. aegilopoides* BAL. — Univ. of Miss. Agr. Exp. Sta. Res. Bull. 248: 1—38.

118. — 1939. Mutant and linkage studies in *Triticum monococcum* and *Tr. aegilopoides*. — Univ. of Miss. Agr. Exp. Sta. Res. Bull. 298: 1—26.

119. — 1941. An inversion, a reciprocal translocation, trisomics, and tetraploids in barley. — Journ. Agr. Res. 63: 741—750.

120. — 1942. Hereditary susceptibility to X-ray injury in *Triticum monococcum*. — Amer. Journ. of Bot. 29: 189—191.

121. — 1943. Relation of polyploidy to heat and X-ray effect in the cereals. — Journ. of Hered. 34: 131—134.

122. STADLER, L. J. 1928 a. Mutations in barley induced by X-rays and radium. Science 68: 186—187.

123. — 1928 b. The rate of induced mutation in relation to dormancy, temperature, and dosage. — Anat. Rec. 41, p. 97.

124. — 1929. Chromosome number and the mutation rate in *Avena* and *Triticum*. — Proc. Nat. Acad. Sci. Washington 15: 876—881.

125. — 1930. Some genetic effects of X-rays in plants. — Journ. of Hered. 21: 2—19.

126. — 1931. The experimental modification of heredity in crop plants. II. Induced mutation. — Sci. Agr. 11: 645—661.

127. STADLER, L. J. 1932. On the genetic nature of induced mutations in plants. — Proc. Sixth Int. Congr. Genet. 1: 274—294.

128. STANTON, T. R. 1923. Prolific and other dwarf oats. — Journ. of Hered. 14: 301—305.

129. STEVENSON, F. J. 1928. Natural crossing in barley. — Journ. Amer. Soc. Agron. 20: 1193—1196.

130. SWENSON, S. P. 1940. Genetic and cytologic studies of a brachytic mutation in barley. — Journ. Agric. Res. 60: 685—713.

131. TAMMES, T. 1925. Mutation und Evolution. — Zschr. f. ind. Abst.- u. Vererb.-lehre 36: 417—426.

132. TEDIN, H. and O. 1927. Contributions to the genetics of barley. II. The development of the kernel basis and its relation to density. — Hereditas IX: 303—312.

133. THUNAEUS, H. 1946. Nya maltkornsorter genom röntgenbestrålning. — Svensk Bryggeritidskrift 61: 73—83.

134. TIMOFÉEFF-RESSOVSKY, N. W. 1937. Experimentelle Mutationsforschung in der Vererbungslehre. — Dresden. 181 pp.

135. TOKHTUYEV, A. V. 1935. Eine neue Mutation bei Gerste: Fehlen der Wachsschicht auf den Blättern. — Trudy prikl. Bot. i pr. II Genetics, Plant Breeding a. Cytology 9: 361—366. (Cited after Der Züchter 9, 1937, p. 102.)

136. UCHIKAWA, I. 1941. Genetic and cytological studies of speltoid wheat. II. Origin of speltoid wheat. — Mem. Coll. Agr. Kyoto Imp. Univ. 50: 1—64.

137. WARBURTON, C. W. 1919. The occurrence of dwarfness in oats. — Journ. Amer. Soc. Agron. 11: 72—76.

138. WERTZ, E. 1940. Über die Abhängigkeit der Röntgenstrahlenwirkung vom Quellungszustand der Gewebe, nach Untersuchungen an Gersten-Körnern. — Strahlentherapie 67: 309—321, 536—550, 700—711, 68: 136—164, 287—302.

139. VESTERGAARD, H. A. B. 1919. Iagttagelser vedrörende Arvelighedsforhold hos Lupin, Hvede og Byg. — Tidsskrift for Planteavl 26: 491—510.

140. — 1928. Abed Planteavlsstation. Forædlingsarbejdet gennem 25 Aar. — Nyköbing. 79 pp.

141. WINGE, Ö. 1924. Zytologische Untersuchungen über Speltoide und andere mutantenähnliche Aberranten beim Weizen. — Hereditas V: 241—286.

142. ZHEGALOV, S. I. 1920. On the genetics of oats. — All Russian Plant Breed. Rpt. 3: 80.

143. ÅBERG, E. and WIEBE, G. A. 1946. Classification of barley varieties grown in the United States and Canada in 1945. — U. S. Departm. Agric. Techn. Bull. 907: 1—190.

144. ÅKERMAN, Å. 1922. Untersuchungen über eine in direktem Sonnenlichte nicht lebensfähige Sippe von *Avena sativa*. — Hereditas III: 147—177.

145. — 1929. Letalfaktorer hos havre och vete. — Beretn. om Nordiske Jordbrugsforskeres Forenings IV. Kongr., Helsingfors: 594—604.

146. ÅKERMAN, Å. und BADER, M. 1938. Über Kreuzungen zwischen *Avena sativa* und *Avena fatua* und einige Untersuchungen über Fatuoiden. — Zschr. f. Pflanzenzücht. 22: 1—44.

147. ÅKERMAN, Å. and FRÖIER, K. 1941. Studien über eine spontane *chlorina*-Mutation in *Avena sativa*. — Hereditas XXVII: 371—404.

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# A CONTRIBUTION TO THE GENETICS OF MILK GLAND ACTIVITY IN CATTLE

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IN a study of the composition of milk, based on comparisons between a number of twin pairs, it was found that the percentages of protein and of lactose *at fixed levels of percentages of fat* are chiefly genetically determined (BONNIER and HANSSON, 1946). That is to say: in two sets of samples of milk, all of which have equal percentages of fat (or, more correctly, have fat percentages the range of variation of which is only 0,1 %), the differences between the means of the two sets in protein percentage or in lactose percentage are within the limits of random variation if the two sets are taken from two identical twins, whereas, from a statistical point of view, the means of the two sets may be very widely apart if they are taken from two unrelated cows. The studies in question are based on 2245 samples taken from 29 pairs of twins of which 19 are identical, 7 are fraternal and 3 are uncertain as to the nature of the twinships. Table 1, in which some of the statistical figures are given, is an excerpt from Tables 3 and 4 of the article cited. The orders of magnitude of the P's are found by comparing the ratios of the mean squares in question by the error mean square.

In most of the cases the two twins of a pair had been treated or fed differently but, so far, it has not been possible to show any certain influence from this differential treatment on the differences between protein or lactose percentages at fixed percentages of fat.

*The number of genes in action.* — A more searching analysis (still always made by comparisons *within* groups of fat percentages, the grouping unit of which is 0,1 %) of the genetical relationships can, however, be made. The average mean squares, corresponding to the comparisons between the means of two identical twins, 0,0713 for protein and 0,1523 for lactose, may be said to constitute a measure of the average difference an animal would show if it did live two different lives. These mean squares have therefore been used as a common base for comparisons between other mean squares by dividing the latter by the former. Thus each mean square corresponding to the difference of the means of two twins of each separate twin pair has been divided by the above-

TABLE 1. *Analysis of variance of the differences between percentages of protein and of the differences between percentages of lactose at fixed fat percentages.*

| | Degrees of freedom | Protein | | Lactose | |
|---|---|---|---|---|---|
| | | Mean square | P | Mean square | P |
| Between groups of different fat percentages..... | 45 | 4,2830 | | 0,7982 | |
| At fixed fat percentages | | | | | |
| Between twin pair means (= between unrelated animals) | 503 | 0,2288 | $< 0{,}001$ | 0,4043 | $< 0{,}001$ |
| Cow versus twin sister | | | | | |
| identical twins ... | 168 | 0,0713 | $> 0{,}05$ | 0,1523 | $> 0{,}05$ |
| uncertain twins ... | 45 | 0,0765 | $> 0{,}05$ | 0,1882 | Appr. $= 0{,}05$ |
| fraternal twins ... | 82 | 0,1048 | $0{,}01$—$0{,}001$ | 0,1920 | Appr. $= 0{,}01$ |
| Within cows (error) | 1401 | 0,0697 | | 0,1359 | |
| Total | 2244 | | | | |

mentioned figures. These ratios are given in Table 2. Furthermore, twin pairs for which this ratio is less than 1,5 for protein as well as for lactose, 18 in number (13 identical, 3 fraternal and 2 uncertain), have been selected, and the mean squares corresponding to the $\frac{17 \times 18}{2} = 153$ differences between two such twin *pair* means have been computed. As above, these 153 mean squares are divided by 0,0713 for protein and by 0,1523 for lactose and the ratios are collocated in two tables (Table 3 for protein and Table 4 for lactose). Finally, all ratios are classified in groups with a grouping unit of 0,5 and the distributions are computed for the 19 identical pairs, the 7 fraternal pairs and for the 153 twin pair combinations (Table 5, Fig. 1).

Before trying to interpret the tables it may be well to explain some of the figures. If we look at Table 3, we find, for instance, that the ratios are: for pairs 9—11, 1,387, for pairs 9—14, 1,472, thus two moderate ratios. But the ratio for pairs 11—14 is very much larger, 11,397. And there are other cases of a more or less similar kind. It may seem natural to expect that, if we have three pairs, and if the difference

TABLE 2. *Ratios between, on the one hand, the mean squares between the two twin means and, on the other, the average mean square between means of two identical twins (0,0713 for protein and 0,1523 for lactose).*

| No. of twin pair | | Nature of twinship | Ratio | |
|---|---|---|---|---|
| In the barn | For reference to Tables 3 and 4 | | Protein | Lactose |
| 4— 5 | 1 | Identical | 0,738 | 1,344 |
| 8— 9 | 2 | » | 0,400 | 0,405 |
| 106—107 | 3 | » | 0,328 | 0,374 |
| 115—116 | 4 | » | 0,180 | 0,147 |
| 119—120 | 5 | » | 0,829 | 1,175 |
| 121—122 | 6 | » | 1,021 | 0,609 |
| 205—206 | 7 | » | 0,389 | 0,681 |
| 207—208 | 8 | » | 0,586 | 0,541 |
| 209—210 | 9 | » | 1,293 | 0,415 |
| 213—214 | 10 | » | 0,878 | 1,268 |
| 725—726 | 11 | » | 1,282 | 0,899 |
| 818—819 | 12 | » | 0,839 | 0,936 |
| 821—822 | 13 | Uncertain | 0,797 | 1,229 |
| 823—824 | 14 | Identical | 1,397 | 0,693 |
| 736—737 | 15 | Fraternal | 0,881 | 0,721 |
| 807—808 | 16 | » | 1,499 | 0,823 |
| 830—831 | 17 | » | 1,224 | 1,324 |
| 716—717 | 18 | Uncertain | 1,449 | 0,545 |
| 123—124 | | Identical | 2,756 | 1,464 |
| 201—202 | | » | 1,659 | 1,020 |
| 203—204 | | » | 0,958 | 1,883 |
| 215—216 | | » | 0,610 | 2,658 |
| 921—922 | | » | 0,853 | 1,536 |
| 117—118 | | » | 1,793 | 1,389 |
| 906—907 | | Fraternal | 0,637 | 1,456 |
| 915—916 | | » | 2,450 | 1,229 |
| 757—758 | | » | 2,006 | 1,312 |
| 647—648 | | » | 1,060 | 2,104 |
| 15— 16 | | Uncertain | 0,929 | 1,978 |

between the first and the second as well as that between the second and the third is small, then also the difference between the first and the third ought to be small. There are two reasons for this not always being the case. Suppose first that all three pairs were represented only within one and the same group of fat percentages. If the numeric value of the difference between the first and the second pair is $a$, and

TABLE 3. *Ratios between, on the one hand, the mean squares of protein differences between two twin pair means and, on the other, the average mean square between means of two identical twins, 0,0713.*

| Twin pair No. | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0,938 | 2,224 | 6,247 | 1,541 | 2,819 | 0,847 | 2,239 | 1,073 | 1,328 | 2,350 | 0,604 | 0,468 | 3,579 | 1,341 | 1,977 | 3,047 | 2,343 |
| 2 | | 1,751 | 3,899 | 1,984 | 3,846 | 4,059 | 2,257 | 1,471 | 0,956 | 2,659 | 2,557 | 2,548 | 5,783 | 1,966 | 1,638 | 3,464 | 2,601 |
| 3 | | | 1,005 | 2,180 | 2,476 | 0,975 | 5,302 | 3,207 | 2,677 | 1,208 | 2,944 | 2,938 | 3,110 | 3,263 | 5,278 | 4,938 | 5,362 |
| 4 | | | | 4,093 | 2,346 | 2,766 | 9,525 | 3,235 | 3,110 | 2,799 | 6,467 | 7,092 | 9,986 | 6,878 | 7,280 | 7,469 | 10,725 |
| 5 | | | | | 2,347 | 3,493 | 3,985 | 1,158 | 1,149 | 1,418 | 1,700 | 1,624 | 9,677 | 2,053 | 2,340 | 2,640 | 4,555 |
| 6 | | | | | | 1,902 | 5,418 | 1,613 | 2,351 | 2,855 | 3,746 | 2,656 | 5,525 | 3,527 | 2,748 | 2,281 | 4,254 |
| 7 | | | | | | | 6,568 | 2,611 | 1,816 | 2,920 | 2,841 | 1,848 | 1,724 | 2,389 | 2,547 | 4,337 | 3,196 |
| 8 | | | | | | | | 1,822 | 2,802 | 4,685 | 2,453 | 2,493 | 2,254 | 1,941 | 1,760 | 3,158 | 1,354 |
| 9 | | | | | | | | | 1,239 | 1,387 | 4,992 | 0,662 | 1,472 | 0,936 | 1,601 | 1,518 | 1,835 |
| 10 | | | | | | | | | | 0,820 | 1,764 | 1,370 | 4,249 | 1,878 | 1,746 | 2,567 | 2,809 |
| 11 | | | | | | | | | | | 1,998 | 2,498 | 11,397 | 2,821 | 2,130 | 3,403 | 4,477 |
| 12 | | | | | | | | | | | | 0,603 | 3,108 | 1,435 | 1,009 | 1,490 | 1,914 |
| 13 | | | | | | | | | | | | | 3,925 | 0,866 | 0,601 | 1,447 | 1,815 |
| 14 | | | | | | | | | | | | | | 3,764 | 2,198 | 5,669 | 2,462 |
| 15 | | | | | | | | | | | | | | | 0,386 | 1,641 | 0,817 |
| 16 | | | | | | | | | | | | | | | | 1,484 | 0,920 |
| 17 | | | | | | | | | | | | | | | | | 2,652 |

TABLE 4. *Ratios between, on the one hand, the mean squares of lactose differences between two twin pair means and, on the other, the average mean square between means of two identical twins, 0,1523.*

| Twin pair No. | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3,489 | 1,766 | 2,288 | 2,664 | 2,770 | 1,199 | 2,150 | 0,839 | 4,987 | 1,121 | 2,697 | 3,006 | 1,555 | 1,613 | 6,969 | 1,289 | 2,285 |
| 2 | | 0,699 | 0,658 | 1,063 | 0,654 | 2,051 | 0,789 | 0,483 | 1,263 | 1,683 | 0,911 | 0,620 | 2,559 | 1,284 | 7,740 | 2,219 | 1,352 |
| 3 | | | 0,708 | 0,298 | 0,847 | 1,269 | 1,559 | 0,919 | 2,614 | 0,827 | 0,568 | 0,749 | 1,180 | 1,210 | 3,823 | 1,186 | 1,146 |
| 4 | | | | 1,677 | 0,625 | 0,810 | 0,661 | 0,423 | 1,091 | 1,531 | 1,203 | 1,188 | 3,127 | 2,317 | 7,887 | 2,338 | 2,762 |
| 5 | | | | | 1,558 | 1,484 | 1,341 | 0,662 | 2,157 | 1,370 | 1,332 | 1,753 | 2,396 | 1,616 | 9,184 | 1,725 | 1,923 |
| 6 | | | | | | 1,785 | 0,954 | 0,578 | 1,379 | 1,651 | 0,998 | 0,568 | 2,258 | 1,680 | 7,331 | 2,089 | 1,436 |
| 7 | | | | | | | 1,692 | 1,245 | 2,249 | 1,561 | 0,771 | 1,222 | 2,811 | 2,690 | 8,727 | 2,022 | 2,746 |
| 8 | | | | | | | | 0,681 | 0,823 | 1,091 | 1,450 | 1,004 | 3,121 | 2,540 | 7,670 | 1,889 | 2,833 |
| 9 | | | | | | | | | 1,616 | 0,494 | 0,636 | 0,550 | 1,062 | 1,827 | 4,858 | 1,138 | 0,943 |
| 10 | | | | | | | | | | 2,389 | 1,979 | 2,173 | 5,421 | 4,028 | 11,154 | 3,766 | 3,738 |
| 11 | | | | | | | | | | | 1,756 | 1,274 | 1,121 | 1,831 | 6,201 | 1,398 | 1,672 |
| 12 | | | | | | | | | | | | 0,846 | 1,952 | 1,356 | 5,751 | 1,963 | 1,297 |
| 13 | | | | | | | | | | | | | 2,260 | 1,742 | 7,089 | 1,916 | 1,962 |
| 14 | | | | | | | | | | | | | | 1,206 | 3,314 | 0,917 | 1,237 |
| 15 | | | | | | | | | | | | | | | 3,956 | 1,671 | 1,464 |
| 16 | | | | | | | | | | | | | | | | 3,438 | 3,422 |
| 17 | | | | | | | | | | | | | | | | | 1,825 |

TABLE 5. *Grouping of the ratios from Table 2 (19 identical twin pairs and 7 fraternal twin pairs) and from Tables 3 and 4 (153 twin pair combinations = unrelated animals).*

| Range of ratios | Protein | | | | | | Lactose | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of ratios | | | In percent of totals | | | Number of ratios | | | In percent of totals | | |
| | Identical twins | Fraternal twins | Unrelated animals | Identical twins | Fraternal twins | Unrelated animals | Identical twins | Fraternal twins | Unrelated animals | Identical twins | Fraternal twins | Unrelated animals |
| 0,00— 0,49 | 4 | | 2 | 21,0 | | 1,3 | 4 | | 4 | 21,0 | | 2,6 |
| 0,50— 0,99 | 8 | 2 | 13 | 42,2 | 28,6 | 8,5 | 6 | 2 | 29 | 31,6 | 28,6 | 19,0 |
| 1,00— 1,49 | 4 | 3 | 19 | 21,0 | 42,8 | 12,4 | 6 | 4 | 36 | 31,6 | 57,1 | 23,5 |
| 1,50— 1,99 | 2 | | 26 | 10,5 | | 17,0 | 2 | | 31 | 10,5 | | 20,3 |
| 2,00— 2,49 | | 2 | 21 | | 28,6 | 13,7 | | 1 | 16 | | 14,3 | 10,5 |
| 2,50— 2,99 | 1 | | 23 | 5,3 | | 15,0 | 1 | | 11 | 5,3 | | 7,2 |
| 3,00— 3,49 | | | 12 | | | 7,8 | | | 7 | | | 4,6 |
| 3,50— 3,99 | | | 8 | | | 5,2 | | | 4 | | | 2,6 |
| 4,00— 4,49 | | | 6 | | | 3,9 | | | 1 | | | 0,7 |
| 4,50— 4,99 | | | 4 | | | 2,6 | | | 2 | | | 1,3 |
| 5,00— 5,49 | | | 4 | | | 2,6 | | | 1 | | | 0,7 |
| 5,50— 5,99 | | | 3 | | | 2,0 | | | 1 | | | 0,7 |
| 6,00— 6,49 | | | 2 | | | 1,3 | | | 1 | | | 0,7 |
| 6,50— 6,99 | | | 2 | | | 1,3 | | | 1 | | | 0,7 |
| 7,00— 7,49 | | | 3 | | | 2,0 | | | 2 | | | 1,3 |
| 7,50— 7,99 | | | | | | | | | 3 | | | 2,0 |
| 8,00— 8,49 | | | | | | | | | | | | |
| 8,50— 8,99 | | | | | | | | | 1 | | | 0,7 |
| 9,00— 9,49 | | | | | | | | | 1 | | | 0,7 |
| 9,50— 9,99 | | | 3 | | | 2,0 | | | | | | |
| 10,00—10,49 | | | | | | | | | | | | |
| 10,50—10,99 | | | 1 | | | 0,7 | | | | | | |
| 11,00—11,49 | | | 1 | | | 0,7 | | | 1 | | | 0,7 |
| Total | 19 | 7 | 153 | | | | 19 | 7 | 153 | | | |

between the second and third is $b$, then the difference between the first and the third may be either $a - b$ or $a + b$. Suppose the latter to be the case. The mean squares corresponding to the three differences are then $\frac{a^2}{2}$, $\frac{b^2}{2}$ and $\frac{a^2+b^2+2ab}{2}$. This means that if two of the figures in Table 3 or 4 are $\alpha$ and $\beta$ the third may be of the order of magnitude $\alpha+\beta+2\sqrt{\alpha\beta}$. This explains several of the cases, but not all. And,

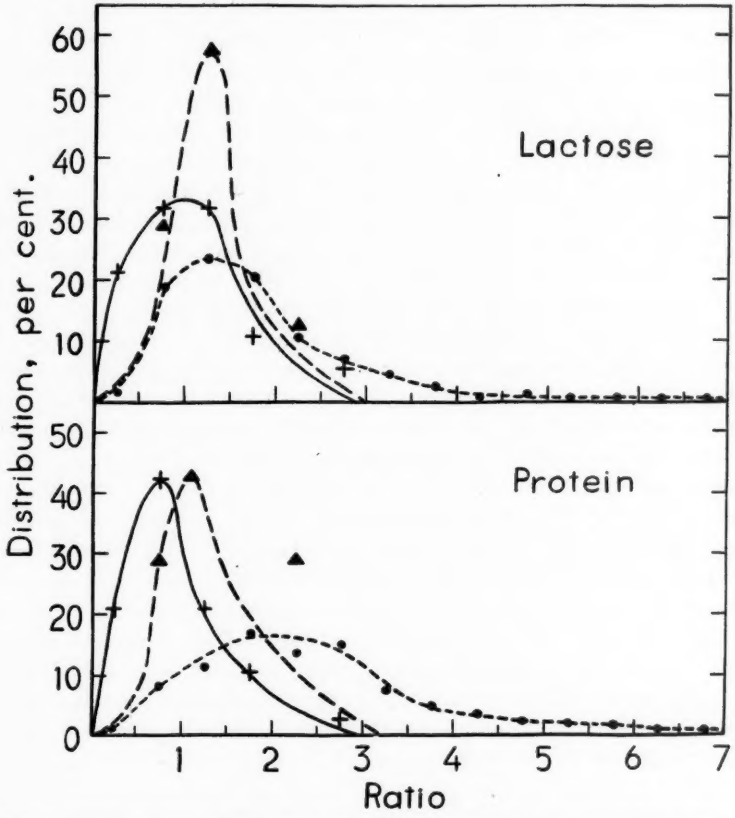


Fig. 1. Graphs corresponding to the distributions of the mean square ratios in Table 5. Crosses = comparisons between identical twins; triangles = comparisons between fraternal twins; dots = comparisons between unrelated animals (= between twin pair means). The curves are fitted by free-hand. (Therefore in the case of fraternal twins, with only 3 groups represented, see Table 5, the fitting is very haphazardous.) For lack of space, the curves for unrelated animals are broken at the ratio-value of 7.

for instance, not the example given, as we have $1{,}387 + 1{,}472 + + 2\sqrt{1{,}387 \times 1{,}472} = 4{,}288$, which is very much less than 11,397. The other reason is random variation. When two pairs are compared, all groups of fat percentages in which both of the pairs are represented will automatically be included in the computation of the mean square of the pair means' difference. But if only one of the pairs (or none) is represented within a certain group of fat percentages, this group is automatically excluded. Thus, if the first and the second pairs are represented within the group of fat percentages 4,00—4,09 but not the third pair, this group contributes to the mean square corresponding to the comparison between the first and the second pair but not to the two remaining comparisons. Likewise, if the second and the third pairs are represented within the group of fat percentages 4,10—4,19 but not the first pair, this group will contribute to the mean square corresponding to the comparison between the second and the third pair but not to the two remaining comparisons. Thus, none of these two groups of fat percentages will contribute to the mean square corresponding to the comparison between the first and the third pair.

Looking now at the distribution of the ratios (Table 5, Fig. 1), it is evident that the range of their variation is smallest for the intra-identical twin comparisons, somewhat larger for the intra-fraternal twin comparisons, and very much larger for the comparisons of twin pair means (i. e. for the comparisons between unrelated animals). This corresponds of course to the data put together in Table 1. But it gives a more apparent and visual picture of the facts. The type of variation in the case of intra-identical twin comparisons must be due only to external (and random) causes. If only one single pair of genes was responsible for the differences between unrelated animals, there would be at most three different values round which the corresponding 153 ratios would vary. And, likewise, if only very few pairs of genes did act, some peaks ought to be found in the curves of distribution of the 153 ratios. But this does not appear to be the case, and thus it seems fair to conclude that several pairs of genes are in action. Hence we are probably concerned here with a case of polygenic effect.

It must be emphasized that the number of genes in action may be still greater, as it is possible that a certain gene difference which is active at one level of fat percentage may be inactive at another level. In such a case other gene differences must be active at that new level.

If the figures of Tables 3 and 4 are compared, it will be found that large ratios in one of the tables do not at all correspond to large ratios

in the other table. Grouping the ratios as in Table 5 with a grouping unit of 0,5 and computing the correlation of the 153 ratios for protein and for lactose, a correlation coefficient of — 0,05 is found. The signification of this is that different — or at least partly different — sets of genes are in action when the percentage of protein and the percentage of lactose are determined at a certain level of fat percentage.

## SUMMARY.

(1) It has been shown in an earlier article that in cow's milk the percentages of protein and of lactose at fixed levels of fat percentages are chiefly genetically determined.

(2) A comparison of the variation in the percentages of protein and of lactose at fixed levels of fat percentages between twin pair means (i. e. between unrelated animals) with the variation between the means of two identical twins has led to the conclusion that several genes must be in action.

(3) It is also shown that different sets of genes are responsible for the percentages of protein and for the percentages of lactose at fixed levels of fat percentages.

## LITERATURE CITED.

1. BONNIER, G. and HANSSON, A. 1946. Studies on monozygous cattle twins. VII. On the genetical determination of the interdependency between the percentages of fat, protein, and lactose in the milk. — Acta Agriculturæ Suecana. (In press.)

# ON THE FORMATION OF BIVALENTS IN SOME PENTATOMIDS (HEMIPTERA)

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DURING a casual examination some ten years ago of preparations showing the spermatogenesis of *Dolycoris baccarum* L., a Hemipteron belonging to the family *Pentatomidae*, the stages of diakinesis particularly attracted my attention. Except in the latest stages of diakinesis no normal bivalents at all were visible, the homologous chromosomes being more or less separated from one another, so that a count of the separate chromosome elements gave the full diploid number of 14 characteristic of this species (12 autosomes, *X* and *Y*). In the late stages of diakinesis and the first metaphase, on the other hand, 6 normal autosome bivalents in addition to the *X* and *Y* chromosomes were visible. It was my intention at the time to embark on a closer investigation of the matter, but other work in progress and the war years then intervening obliged me to postpone the realisation of this intention for some time.

A few years ago, when SCHRADER's investigation on the formation of tetrads in another Pentatomid, *Rhytidolomia senilis* SAY, published as early as 1940 fell into my hands, I found that he had there elucidated the same phenomenon that I had noticed in *Dolycoris*. Since, however, the conditions appearing in *Rhytidolomia* did not, to my mind, correspond in every way with those I found in *Dolycoris*, I determined to carry out a corresponding investigation with respect to *Dolycoris* also.

*Material and methods.* — My material was collected in July, 1945, on the Hanko peninsula in the territory surrounding the zoological station of Tvärminne. In addition to *Dolycoris*, specimens of another, closely related Pentatomid, *Carpocoris purpureipennis* DE G., were also taken. All the specimens of which testes were fixed were in the last larval stage. In this stage all the phases of meiosis are plentifully represented. Spermatogonia, on the other hand, are but seldom found. In the collecting of the material I enjoyed the friendly assistance of Dr. HÅKAN LINDBERG, for which I should like here to express my best thanks.

The material was fixed in BENDA, CARNOY, RANDOLPH and BOUIN-

ALLEN. Of these, RANDOLPH gave the best results, at least as far as the early stages of meiosis were concerned. The microtome sections were made 10 or 15 $\mu$ thick. The preparations were stained with iron haematoxylin, crystal violet, or by the FEULGEN method. No smears were made.

The pictures were drawn with an ABBE drawing apparatus at the height of the work-table, using an immersion objective of 100 × and an ocular of 25 ×. Since the drawings were reduced in size by one-half in making the printing-plates, the magnification is now about 2250 ×.

*Observations.* — In *Dolycoris*, as in so many other Hemiptera, it is impossible to make any observations on the primary pairing of the chromosomes, since the earliest stages of meiosis, the leptotene and presumably also the zygotene stage, are characterized by particularly strong synizesis. The thin chromosome threads are drawn together into a single tangle in the centre of the nucleus (Fig. 1), a typical central synizesis. When the conditions causing synizesis have passed, the close tangle of chromosomes loosens and begins to expand in every direction, tending to fill evenly the whole nucleus. In this stage the chromosomes are comparatively thick, strongly stained threads (Fig. 2).

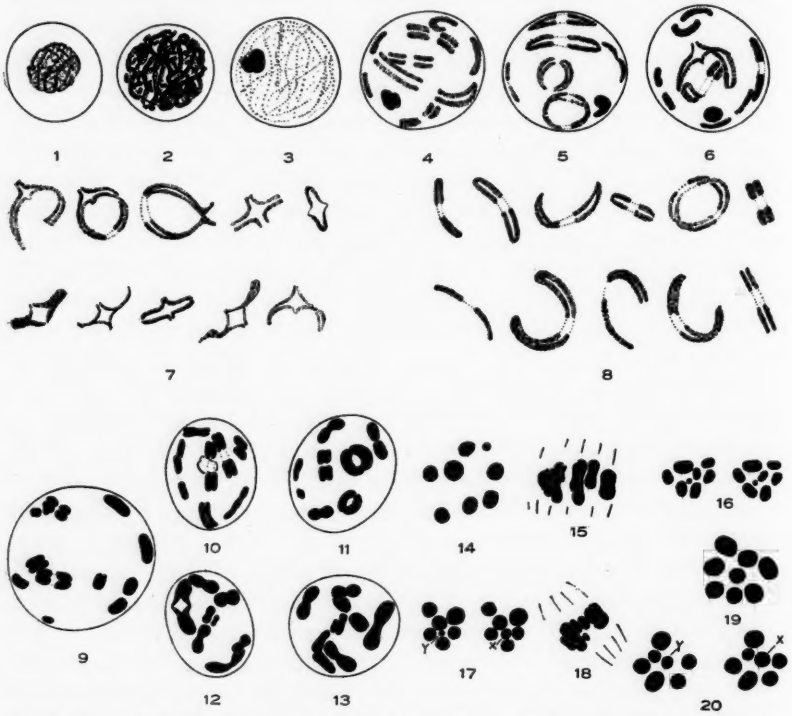
This pachytene stage does not, however, seem to be of long duration, the change to the diffuse stage taking place immediately all the signs of true synizesis have disappeared (Fig. 3). The chromosomes now lose their ability to stain almost completely, making it impossible to find out anything about their structure. This stage is also accompanied by a very appreciable increase in size of the nucleus. In contradistinction to the autosomes, which are entirely diffuse, the sex chromosomes appear in this stage as strongly stained, compact bodies. The *X* and *Y* chromosomes here show a strong tendency to associate (cf. GEITLER, 1939, p. 216). They are on this account generally visible either as a sort of doublet, in which the smaller *Y* appears just as if it were but a bulge on the side of the larger *X* (Figs. 3—5), or entirely fused together, leaving only a single, spherical and apparently homogeneous body (Fig. 6). The close packing of the chromosomes makes it impossible to decide whether the sex chromosomes are heteropycnotic already during the synizesis and early pachytene stages.

No nucleolus, or at least no discernible one, appears during the prophase of meiosis, for no other definite, compact body other than the *XY* complex is to be observed during these phases. Since the sex chromosome complex appears exactly the same in both FEULGEN and

crystal violet preparations, it cannot be accompained by a nucleolus.

As it is impossible to ascertain anything about the pairing relations and chiasma formation of the chromosomes before or during the diffuse stage, the stage when the chromosomes again become visible after the diffuse stage is of particular importance for the investigation of bivalent formation.

Characterizing the stage in question in *Rhytidolomia*, SCHRADER (1940) mentions that the diploid number of chromosome elements




Figs. 1—18. *Dolycoris baccarum*. — Fig. 1, synizesis (leptotene stage). — Fig. 2, pachytene stage. — Fig. 3, diffuse stage. — Figs. 4—6, early diakinesis. — Fig. 7, bivalents with typical not terminalised chiasmata in early diakinesis. — Fig. 8, bivalents with »super-terminalised distance chiasmata» in early diakinesis. — Figs. 9—13, late diakinesis. — Fig. 14, first metaphase plate. — Fig. 15, first metaphase plate in side view. — Fig. 16, sister plates of first anaphase. — Fig. 17, second metaphase; the same plate in two different levels. — Fig. 18, second metaphase in side view. — Figs. 19 and 20, *Carpocoris purpureipennis*. — Fig. 19, first metaphase plate. — Fig. 20, second metaphase, the same plate in two different levels.

occurs and that the homologous chromosomes are entirely isolated from one another, there being no visible connections and even considerable intervals between them. Thus, he affirms (1940, p. 127) that the condition in question is one with no sign of »parasynaptic pairing» or chiasma formation.

The situation in *Dolycoris* is largely analogous (Figs. 4—6). There is very often the full diploid number of 12 autosome elements. Their relative position, however, is nearly always such as to make it easy to see which of them are paired together, even though definite morphological differences do not exist between all of the chromosomes. Very often, though not always, a very thin thread connects homologous chromosomes, which shows that we nevertheless have bivalents, notwithstanding the fact that the connection between their two components is extremely weak. The greater the distance between the homologues the weaker this connection thread generally is, and in extreme cases there appears, as stated above, to be no connection at all (Fig. 4). Inasmuch, however, as also in these cases the topographical relations between the chromosomes are such as to suggest some bond connecting the homologues, even though it is invisible, it seems to me very possible that such a bond actually always exists, though it can no longer take stain on account of its thinness.

In some cases, however, the homologues are not separated from one another but form typical end-to-end pairs. Finally, I have in some cases observed figures showing entirely clear and typical chiasmata (Figs. 6 and 7). Mostly these chiasmata are already very near complete terminalisation, though sometimes they still have a good distance to wander. Usually there is only one chiasma for each bivalent, but in the larger bivalents two chiasmata are also often found, terminalising on opposite ends. In rare cases one of the smaller bivalents may also form two chiasmata.

It is, thus, impossible to observe directly what occurs during the diffuse stage, comprising the latter part of the pachytene and most of the diplotene stage. But the fact that chiasmata are present at all, even though they are few, in the stages immediately following the diffuse stage gives indications that help to solve the question and throw a decisive light on the problem of bivalent formation in *Dolycoris*. For, in my opinion, it is quite apparent that chiasmata have formed normally and in the usual manner during the diffuse stage but have in the main had time to terminalise before becoming chromatinized sufficiently to be visible. Only a few have been so long delayed in terminalisation

that interstitial chiasmata may still be observed when the chromosome becomes visible.

The prophase of meiosis in *Dolycoris* is, then, characterized by rapid progress of chiasma terminalisation compared to the progress of chromatinisation and contraction of the chromosomes. When the chromosomes become visible after the confused stage, this latter development has reached a degree generally characterizing the diplotene stage, whereas terminalisation has in most cases progressed not only to the maximum degree, attained generally only in late diakinesis, but even beyond that, going over into a kind of »super-terminalisation» manifesting itself in a moving apart of the chromosome partners, often very far indeed from one another, the terminal connection between them stretching to a very thin thread.

The changes that the chromosomes thereafter pass through in the last stages of the meiotic prophase (Figs. 9—13), that is to say, up to the metaphase of the first maturation division, are founded on their continued chromatinisation and contraction, in addition to which the halves of each bivalent approach each other. In some cases the contraction and chromatinisation have attained almost their maximum degree even though the homologous chromosomes are still widely separated (Figs. 9 and 10), but generally they approach each other in the same measure as chromatinisation and contraction progress. Sometimes, it is true, thin connecting threads may be observed between the homologues that are approaching each other, like the threads that sometimes appear already in early diakinesis, but often there is no bond until the compact chromosomes have attained direct contact.

This final formation of bivalents generally takes place by an association of the homologous chromosomes at only one end. In the case of the large autosome pair, however, this association comparatively often takes place at both ends, forming a ring (Fig. 11). Thus, the large autosome appeared as a ring in 14 (or 14,9 %) of the 94 diakinesis nuclei examined. Smaller autosomes cannot be identified, but in 5 out of a total of 54 cells I was able to ascertain that one of the smaller autosome pairs formed a ring (Fig. 11).

The maturation divisions (Figs. 14—18) excite no particular comment, for they are in entire accord with corresponding stages in other Hemiptera. *X* and *Y* do not pair in the first division even though they generally lie side by side or at least quite close to one another in diakinesis, probably due to their close association in the early stages of meiosis. Thus, 8 separate chromosome elements are always visible in

the metaphase plate of the first division, which means that *X* and *Y* divide equationally in the first division. There is practically no interkinesis, the first division anaphase plates (Fig. 16) going over directly into the second maturation division. *X* and *Y* show a tendency to pair already during the first anaphase, and in the second maturation division (Figs. 17 and 18) they then move to different poles.

The circumstances at bivalent formation in *Carpocoris purpureipennis*, the other Pentatomid examined, correspond to the above in every way. It is also in other respects karyologically very similar to *Dolycoris* (Figs. 19 and 20). Thus, it has the same number of chromosomes, and the sex chromosomes behave in a corresponding manner. However, the *Y* chromosome is much larger in size than in *Dolycoris*, being about as large as the smallest of the autosomes (Fig. 20).

*Discussion.* — Though the process of bivalent formation in *Dolycoris* seems at first glance to depart radically from the normal, the facts presented above show that there is really no fundamental difference. *The most important departure from normal lies in the exceptionally early terminalisation of the chiasmata*, which, as already mentioned, appears in the main to take place already during the diffuse diplotene stage.

Besides being earlier, terminalisation is also more complete than usual. This fact confirms my statement earlier made in another connection (OKSALA, 1943, p. 21) to the effect that the earlier — relatively to contraction — terminalisation takes place the more completely it develops. It is but natural that it should be easier for the chiasmata to move when the chromatids composing the bivalent are still thin and weakly spiralled, and not burdened with a great amount of thymonucleic acid either. Thus terminalisation does, in fact, quite apparently take place in *Dolycoris* more easily than normal, for it attains its maximum degree very quickly and usually even exceeds it, for I interpret the moving apart of the bivalent halves in early diakinesis more as a sort of »super-terminalisation» than as true breakage of chiasmata and break-down of the bivalents. This conception of the matter may, in my opinion, be defended on the following grounds.

A visible though thin bond often persists between the halves of the bivalents all through diakinesis. This being the case, we are justified in saying that the chiasma still exists. And even when there is no visible bond the position of the homologous chromosomes relative to one another is such as to give a strong impression of their belonging together. As far as I can see, we may well consider an actual bond to

exist, even though too thin to stain. Also, the manner in which the bivalents attain their final shape in the last stages of diakinesis speaks for retention of the chiasmata. The attraction between homologous chromosomes at this stage is remarkable inasmuch as it generally unites the small autosomes at only one end, the largest autosome pair, however, either at one or at both ends. Herein there appears a clear analogy to chiasma formation, for in the smaller autosomes there is generally formed but one chiasma, terminalising on one end, whereas the largest autosome may also form two chiasmata, thus often appearing as a ring. If, now, this terminal attraction appearing in the last stage of diakinesis were a phenomenon entirely independent of previous chiasma formation, it would be incomprehensible why it should take place in the manner just described. Why should it not then unite all chromosomes at both ends or all at only one end? Actually, however, the original chiasmata are quite evidently renewed. *Thus we have reason to presume that the terminal chiasmata have at no stage completely disappeared, but much rather have super-terminalised and stretched exceedingly thin, yet been retained as effective »distance chiasmata» all throughout diakinesis.*

The phenomena here remarked in *Dolycoris* are, in my opinion, of a nature to throw new light in some respects also on the case of *Rhytidolomia* described by SCHRADER. This inasmuch as meiosis in these two species shows so much in common that it appears to me justifiable to interpret both cases on the same basis. Thus, *Dolycoris* may in respect to bivalent formation be considered as a sort of intermediate form between normal Hemiptera and *Rhytidolomia.* The tendency already clearly observable in *Dolycoris* has evidently attained its peak in *Rhytidolomia.* On these grounds it would seem to me to be not too daring to assume more or less normal chiasma formation to occur in *Rhytidolomia* also, even though this as well as the terminalisation of the chiasmata takes place entirely during the diffuse stage. In *Dolycoris,* on the other hand, the last vestiges of chiasmata are still perceptible when the chromosomes reappear clearly defined after the diffuse stage. An effective bond between homologous chromosomes throughout diakinesis quite apparently exists also in *Rhytidolomia,* to judge from SCHRADER's illustrations (the microtome sections only are to be taken into account in this connection), and the selective end-to-end attraction between these chromosomes apparent in the last stage of diakinesis would, in *Rhytidolomia* as in *Dolycoris,* be unexplainable were it independent of primary chiasma formation. The final formation

of bivalents at the close of diakinesis need, therefore, in no wise be considered the result of some entirely new »pairing effect». On the contrary, it may simply be a question of the familiar phenomenon that on entering into the metaphase of the first maturation division the bivalent halves contract to the limit and cling to each other more closely.

To my mind *there is therefore no reason to consider Rhytidolomia as unique in respect to bivalent formation any more than Dolycoris and Carpocoris are.*

In conclusion I cannot desist from saying a few words concerning bivalent formation in another Hemipteron, *Alydus calcaratus* L. (*Coreidae*). REUTER, who has described the whole development here with great exactitude, remarks that there is nothing that would indicate segment exchange (nor, therefore, chiasma formation). The original, typical parallel pairing simply changes over into end-to-end pairing at a certain stage. Since the diffuse stage is not so marked, and inasmuch as all the phases of spermatogenesis are clearly to be observed, step by step, there should be no question of a veiled chiasma formation such as we found in *Dolycoris.* Still, it is not impossible that the bivalents arise on the basis of chiasma formation in *Alydus* also, if we consider the chiasmata as localized out to the extreme end of the chromosomes. Some of the illustrations shown by REUTER (e. g., Figs. 45 and 83) seem clearly to speak for such an interpretation.

## SUMMARY.

The investigation concerns bivalent formation during spermatogenesis in two members of the family of *Pentatomidae*, *Dolycoris baccarum* and *Carpocoris purpureipennis.*

In most cases terminalisation is completed during the so-called diffuse stage, and in consequence non-terminalised chiasmata are extremely seldom visible when the chromosomes become clearly defined in early diakinesis. The chromosomes composing each bivalent have at this point moved apart, often even very far from one another, so that the diploid number of chromosomes is often visible in the nucleus. Sometimes the bivalent halves are united by a thread stretched very thin, sometimes again no bond is visible, which may possibly be explained as due to these threads being too thin and fine to stain. In any case it is apparent that some effective connection exists between the bivalents halves. The true terminal chiasma has thus through

»super-terminalisation» changed into a »distance chiasma». At the end of diakinesis the homologous chromosomes finally move into close contact, and the bivalent attains its final form.

In the opinion of the author, the formation of bivalents in *Rhytidolomia senilis*, investigated by SCHRADER, must also be interpreted on the same principles, i. e. assuming the chiasma to persist all through diakinesis. Thus all of these Pentatomids differ from the normal only in earlier and more complete terminalisation of chiasmata in time relation to chromosome contraction and chromatinisation during the prophase of meiosis.

## LITERATURE CITED.

1. GEITLER, L. 1939. Das Heterochromatin der Geschlechtschromosomen bei Heteropteren. — Chromosoma 1, pp. 197—229.
2. OKSALA, T. 1943. Zytologische Studien an Odonaten. I. Chromosomenverhältnisse bei der Gattung *Aeschna* mit besonderer Berücksichtigung der postreduktionellen Teilung der Bivalente. — Ann. Acad. Sc. Fenn., A, IV, 4, pp. 1—64.
3. REUTER, E. 1930. Beiträge zu einer einheitlichen Auffassung gewisser Chromosomenfragen mit besonderer Berücksichtigung der Chromosomenverhältnisse in der Spermatogenese von *Alydus calcaratus* L. (*Hemiptera*). — Acta Zool. Fenn., 9, pp. 1—487.
4. SCHRADER, F. 1940. The formation of tetrads and the meiotic mitoses in the male of *Rhytidolomia senilis* SAY (*Hemiptera Heteroptera*). — Journ. of Morph., 67, pp. 123—141.

# TOTALE INVENTIERUNG DER MIKROTYPEN EINES MINIMIAREALS VON TARAXACUM OFFICINALE

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(With a Summary in English)

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DIE grosse Variabilität der Gattung *Hieracium* ist lange bekannt. Schon DARWIN nannte sie eine protëische Gattung. NÄGELI und PETER haben grosse Mühe auf eine Aufklärung der mitteleuropäischen Formenkreise verwendet und DAHLSTEDT und viele andere nordische Forscher bezüglich der nordischen. Gewisse nordische Hieracien (vor allem *Archieracia*) sind hierbei ganz ausserordentlich aufgespaltet worden. Man rechnet schon mit mehr als 2.000 »Mikrospezies». Andere Formenkreise hat man nur teilweise oder gar nicht einer derartigen »Pulverisierung» unterwerfen können. Die Ursache ist nunmehr klar. Es ist hierfür der Grad der agamospermen Samenbildung entscheidend. Nur bei totaler Agamospermie ist eine Unterscheidung bestimmter Formentypen möglich, bei partieller ist dies kaum und bei sexueller Befruchtung gar nicht durchführbar.

Zu Beginn dieses Jahrhunderts wurde durch DAHLSTEDT die Polymorphie von *Taraxacum officinale* entdeckt. Diese Population hat man bis 1900 als *eine* einzige Spezies betrachtet und innerhalb dieser sind nur einige wenige Varietäten unterschieden worden, die hauptsächlich auf extreme Standorte begrenzt waren. LINNÉ führte *Taraxacum* nicht einmal als eigene Gattung auf. *T. officinale* wurde von ihm der Gattung *Leontodon* zugerechnet und *Leontodon Taraxacum* genannt.

Während den letzten Dezennien haben mehrere nordische Forscher, ausser DAHLSTEDT vor allem LINDBERG FIL., MARKLUND und HAGLUND, eine gründliche Untersuchung der *Taraxaca* des Nordens vorgenommen. Die Anzahl der von ihnen aufgestellten Arten beträgt nach einer Zusammenstellung von HYLANDER (1941) nicht weniger als 450. Natürlich entsteht bei dieser Artenfülle sogleich die Frage: Hat man damit die ganze Anzahl der unterscheidbaren Typen erreicht? Und weiter: Können diese Arten überhaupt auseinander gehalten werden? Gibt es zwischen ihnen nicht noch weitere Formen, die ihre Unterscheidung

erschweren oder unmöglich machen? Ist die Anzahl der erblichen Formen, der Biotypen, nicht so gross, dass es ganz aussichtslos wäre, eine Aufteilung in Kleinarten, Mikrospezies, zu versuchen?

Die nordische Population der *Taraxacum officinale* ist für eine Untersuchung des Formenreichtums jedenfalls von einem Gesichtspunkt aus besonders geeignet; sie bildet nämlich stets Samen ohne Befruchtung, zeigt also *vollkommene* Agamospermie. Unter den Formen vom nördlichsten Skandinavien bis nach Dänemark hat man keine einzige sexuelle oder partiell sexuelle Form gefunden. (Ein einziger bei Gothenburg angetroffener Typus, *T. obtusilobum*, ist aller Wahrscheinlichkeit nach eingeführt.) Die *Konstanz* der Typen ist deshalb eine *vollständige*. Eine *Neuerzeugung* von Formen durch Kreuzung und Spaltung erscheint daher *ausgeschlossen*. Das ist ein wichtiger Ausgangspunkt.

Nun fragt man also: Ist die Variabilität überhaupt erschöpfbar? Kann die Anzahl der Biotypen, obgleich sehr gross, durch das hoch entwickelte Unterscheidungsvermögen des Spezialisten wirklich festgestellt werden?

Das ist eine Frage, die taxonomisch kaum zu beantworten ist. Denn der Systematiker unterscheidet ja immer Durchschnittstypen. Unter einer bestimmten Bezeichnung, Beschreibung, fasst er *Ähnliches* zusammen. Ob die Differenzen gross oder klein sind, ob er mit Arten oder Formen arbeitet, immer ist sein Auge das Werkzeug und ein Durchschnittstypus das Mass. Das schon Unterschiedene *sucht er auf* und findet hierbei auch Neues. Ob aber kleinere Abweichungen als die durch die »Kleinarten» bestimmten da sind, dafür hat er kein Interesse; ganz natürlich deshalb, weil er dies nicht entscheiden kann.

Für die Entscheidung, ob die unterschiedenen Typen von *Taraxacum* nur kleinere Durchschnittstypen sind um die noch eine geringere Variabilität zu finden ist, oder ob sie eine reduzierte Anzahl von Biotypen darstellen, zwischen denen es keine Zwischenformen gibt, muss das Experiment herangezogen werden.

Man hat auch Kulturversuche vorgenommen. Hierbei haben sich die Formen als ganz konstant erwiesen. Bei unsrer heutigen Kenntnis ihrer Fortpflanzungsweise ist das ja selbstverständlich. Sie sind apomiktisch konstant durch ihre agamosperme Samenvermehrung, gerade so wie Pflanzen, die durch Bulbillen oder Stecklinge vermehrt werden, konstant sind. Da man ferner die *in der Natur als verschieden konstatierten* Formen in der Kultur geprüft hat, hat man über die *Totalvariabilität* keinen Aufschluss erhalten.

Um die Frage nach der totalen Variabilität, dem Biotypeninhalt

von *Taraxacum*, beantworten zu können, ist eine ganz andere experimentelle Anordnung erforderlich, bei der die zu untersuchenden Individuen *nicht ausgelesen*, sondern bei der *alle Individuen* eines Areals in den vergleichenden Kulturversuch gelangen.

Ich ging in folgender Weise vor. An Standorten mit einer reichen *Taraxacum*-Flora beutelte ich mit Tülltüten die Köpfchen *aller* Pflanzen ohne Ausnahme ein, *ob sie nun verschieden erschienen oder nicht.* Die Tülltüten sollten nur die Samen bei der Reife zurückhalten. Dicht schliessende Pergamintüten waren nicht notwendig, da keine gegenseitige Befruchtung stattfindet.

Zwecks Einsammlung wurden drei verschiedene Standorte gewählt. Von einem Grasrand zwischen den Bäumen einer Allee in Alnarp nahe Lund, der mehr als 50 Jahre unberührt geblieben war, wurden sämtliche Pflanzen einer Fläche von 5 qm eingebeutelt. Hier hatten die *Taraxaca* offenbar eine starke Konkurrenz mit den übrigen Pflanzen des Bestandes, hauptsächlich Gräsern, auszustehen. Überdies war der Bestand jährlich zweimal gemäht worden.

Nur 50 m von diesem Standort entfernt wurde ein weiterer von 5 qm für die Versuche gewählt. Er lag an einem Wegrand, ganz offen. Ein Bestand von Gramineen befand sich in Entwicklung, war aber noch nicht ganz geschlossen. Die Konkurrenz der *Taraxaca* war hier sehr viel geringer als im vorigen Bestand.

Fast 1 km von diesem Standort entfernt befand sich an einem Weg, der von einer Hecke gegen den Park von Alnarp abgegrenzt ist, eine sehr reiche *Taraxacum*-Flora. Der Standort lag sonnenoffen. Der Wegrand wird von Unkraut frei gehalten, weshalb die blühenden Pflanzen sich dort im vergangenen Herbst angesiedelt haben müssen. Offenbar stammten sie nicht nur von Pflanzen, die voriges Jahr dort gewachsen sind, sondern auch von Samen in der Gegend wachsender Pflanzen, die an der Hecke am Weiterfliegen gehindert worden sind und sich am Heckenrand angesammelt hatten. Die Pflanzen müssen hier deshalb zum grössten Teil als hemerophile Ansiedler betrachtet werden. Daher war an diesem Standort auch der grösste Formenreichtum zu erwarten. Da dies für die Untersuchung bedeutungsvoll war, wurden die Pflanzen eines grösseren Areals als an den obengenannten Stellen, nämlich von 40 qm, eingebeutelt. Insgesamt wurden also die Samen sämtlicher Pflanzen eines Gesamtareals von 50 qm für die weitere Untersuchung eingesammelt.

Im Vorsommer desselben Jahres (1934) wurden die Samenproben, 177 an der Zahl, in kleine Kästen ausgesät. Die Erde wurde nicht

sterilisiert, aber einer Tiefe entnommen, in der während des letzten Jahrzehntes keine Umgrabung stattgefunden hatte. Wenn hier überhaupt *Taraxacum*-Samen vorkamen, so konnten sie das Experiment nicht stören, da sie ihre Keimfähigkeit schon nach 4—5 Jahren verlieren. Es konnten auch während der ganzen folgenden Versuchszeit (bis 1940) keine Einmischungen festgestellt werden.

Früh im Herbst (August) wurden die Pflanzen im Botanischen Garten zu Lund auf Freiland ausgepflanzt. Jede Nummer, also jede Nachkommenschaft, bildete eine Parzelle mit 15 Pflanzen. Die Parzellen lagen ganz und gar in der bei dem Einsammeln benutzten Reihenfolge. Jeder Versuch einer Vereinigung ähnlicher Ausgangspflanzen oder ähnlicher Nachkommen in den Kästen wurde vermieden. Erst nachdem die Nachkommen auf dem Felde standen, nebeneinander und übersichtlich, wurde die *Beurteilung der Variabilität* vorgenommen.

Mit dieser vollständigen Inventierung eines Minimiareals habe ich also eine Methode benutzt, durch die die Variabilität voll und ganz beibehalten worden ist. Durch die Parzellen wird jede Ausgangspflanze multipliziert und alle Nachkommen werden auf einen begrenzten Raum vereint, sodass eine vergleichende Übersicht erhalten wird. Erst hierdurch wird es möglich, auch kleinere Differenzen festzustellen.

Schon im Rosettenstadium fiel es auf, dass die Parzellen keine kontinuierliche Variabilitätsreihe bildeten. Gegenüber einem ähnlichen Versuch mit Kulturpflanzen, mit dessen Verhalten ich durch meine frühere Züchtungsarbeit sehr vertraut war, war sogleich ein ganz bestimmter Unterschied zu erkennen. Hat man eine Parzellenreihe von Nachkommenschaften einzelner Pflanzen, so bilden diese eine fast kontinuierliche Serie, binnen der die Varianten keineswegs in Gruppen vereint werden können. Sogar bei selbstbefruchtenden Arten, wie z. B. mit Hafer, erhält man bei der Züchtung einer Landsorte dieses Resultat. Dies beruht natürlich darauf, dass es noch so viele Biotypen gibt, dass zwischen ihnen keine Lücken erkennbar sind. Das Auge hat für ein Unterscheiden keine Anhaltspunkte. Alles fliesst. Wählt man zu einer derartigen Auslese einen Fremdbefruchter, wie z. B. Roggen, so wird die Variabilität natürlich noch kontinuierlicher.

Für *Taraxacum* war es, als die Rosetten im Spätherbst vollausgebildet waren, nicht schwierig, die Parzellen in bestimmte Gruppen zu vereinigen. Noch deutlicher traten die Unterschiede im folgenden Jahr, nachdem die Blüte eingetreten war, zutage. Innerhalb der Parzellen waren alle Pflanzen hochgradig übereinstimmend. Zu einer bestimmten Gruppe konnte ich in einigen Fällen viele Parzellen, die ganz

übereinstimmend erschienen, vereinigen, in anderen Fällen nur wenige, und schliesslich bildete in gewissen Fällen eine einzige Parzelle eine Gruppe. In dieser Weise erhielt ich 24 Gruppen. Von diesen waren indessen 6 anderen Gruppen sehr ähnlich und wichen nur in einzelnen Eigenschaften von diesen ab. Eine Parzelle war z. B. so auffallend später als der Typus, dem sie sonst anzugehören schien, dass die Differenz zwei Wochen betrug.

Es galt nun festzustellen, ob die letztgenannten Abweicher wirklich erbliche Kleinformen oder nur reine Modifikationen darstellten. Für die erste Alternative schien die Tatsache zu sprechen, dass alle Individuen der abweichenden Parzelle in derselben Richtung variierten.

Auch dieses Jahr wurden von allen Parzellen Pflanzen eingebeutelt, sodass Samen für weitere Versuche erhalten wurden und der ganze Versuch demnach wiederholt werden konnte. Beim Auspflanzen der neuen Parzellen im Herbst 1935 wurden die Gruppen übereinstimmender Parzellen vereinigt, sodass diese unter sehr ähnlichen Substratbedingungen wuchsen und so genauer miteinander verglichen werden konnten.

Schon dieses Jahr erwiesen sich von den erwähnten 6 Gruppen 4 als nicht oder nicht sicher abweichend. Die erwähnte sehr spätblühende Parzelle hatte dieses Jahr ganz dieselbe Blütezeit wie die Parzellen seines Typus. Offenbar war ihre Abweichung von rein modifikativer Natur. Überhaupt scheint die modifikative Variabilität bei *Taraxacum* eine ganz beträchtliche zu sein ohne aber die Grenzen zwischen den Typen unter gleichartigen Kulturverhältnissen zu verwischen.

Zwei der 6 Gruppen schienen dagegen untereinander und von den während des vorigen Jahres unterschiedenen deutlich abweichend zu sein.

Der ganze Versuch wurde 1936 noch einmal durch Samenanbau wiederholt, weil ich eine ganz sichere Beurteilung der Gesamtvariabilität erhalten wollte. Das Resultat war mit dem des vorigen Jahres vollkommen übereinstimmend. Die Parzellen konnten ganz scharf in 20 (18 + 2) Gruppen aufgeteilt werden.

Im Jahre 1938 fand nur eine teilweise Wiederholung statt. Die oben erwähnten 4 wahrscheinlich modifikativen Gruppen, die in bezug auf Pigmentierung, Rosettenfarbe, Zerschlitzung des Blattrandes oder Form und Richtung der Hüllblätter von ihren Gruppen abzuweichen schienen, wurden durch zwei Wiederholungen sehr sorgfältig geprüft, da es wichtig war festzustellen, ob ihre Abweichung, die ganz kontinuierlich erschien, von Jahr zu Jahr und in den verschidenen Wiederholungen ein und desselben Jahres in dieselbe Richtung gehen würde.

Auch einige Parzellen, die während den früheren Jahren eine diminutive Abweichung von ihren Gruppen gezeigt hatten, wurden in derselben Weise weiter geprüft.

Auch 1939 wurde ein Teilversuch in gleicher Umfassung und gleicher Anordnung wie 1938 ausgeführt.

Das Schlussresultat der ganzen, während 5 Generationen fortgesetzten Untersuchung war, dass sämtliche Nachkommen der Ursprungspflanzen sich auf 20 verschiedene Typen verteilten. Die anfangs als kleinere Varianten vermuteten Abweicher erwiesen sich *alle* als nicht erblich, waren demnach reine Modifikationen. Sie schwankten während den Versuchsjahren zwischen den Plus- und Minusgrenzen der Modifikabilität eines bestimmten Typus.

Die Unterschiede zwischen den Typen (»Mikrospezies») waren mehr oder weniger ausgeprägt, *immer* aber deutlich, distinkt. Seitdem ich die Nachkommen der Ausgangspflanzen in Parzellen nebeneinander hatte, war ich in bezug auf ihre Aufteilung in eine Anzahl ganz bestimmter und definitiver Gruppen ganz im klaren. Es gab 20 Gruppen, zwanzig erblich verschiedene Typen.

Ich nannte diese Typen schlechtweg Nr. 1—20. Sie unter den schon beschriebenen 450 »Kleinspezies» aufzusuchen und mit gewissen dieser zu identifizieren wäre gewiss eine sehr mühsame Arbeit gewesen.

Es war für mich daher von grossem Vorteil, dass meine Typen von dem bekannten Taraxakologen GUSTAF HAGLUND durchgesehen und identifiziert wurden. Ich benutze die Gelegenheit ihm auch an dieser Stelle hierfür meinen herzlichen Dank zu sagen.

Folgende Typen wurden in meinen Versuchen festgestellt (auch die Anzahl der Parzellen jedes Typus, also seine Gesamtfrequenz an dem Minimiareal in der Natur wird angegeben):

| | | | |
|---|---|---|---|
| *pycnolobum* DT. | 51 | *aequilobum* DT. | 1 |
| *sagittatum* DT. | 29 | *Dahlstedtii* LINDB. FIL. | 1 |
| *retroflexum* LINDB. FIL. | 26 | *decurtatum* nov. | 1 |
| *polyodon* DT. | 14 | *dilatatum* LINDB. FIL. | 1 |
| *brevisectum* PALMGR. | 13 | *duplidens* LINDB. FIL. | 1 |
| *angustisquameum* DT. | 11 | *fulvum* RAUNK. | 1 |
| *caudatulum* DT. | 7 | *laeticolor* DT. | 1 |
| *cordatum* PALMGR. | 7 | *longisquameum* LINDB. FIL. | 1 |
| *sublaeticolor* DT. | 4 | *laeticeps* HAGL. | 1 |
| *mimulum* DT. | 4 | | Summe 177 |
| *xanthostigma* LINDB. FIL. | 2 | | |

Alle Pflanzen ausser einer einzigen konnten mit früher beschriebenen »Mikrospezies» identifiziert werden. Diese einzige ist die *decurtatum* nov. genannte. Sie stand dem *pycnolobum*-Typus nahe, wich aber in mehreren Eigenschaften, die sich durch alle der 5 Generationen schön wiederholten, ab. Schon die junge Rosette wich von *pycnolobum* durch ihre dem Boden angedrückten dunkelgrünen Blätter ab. Die *pycnolobum*-Rosette hat etwas aufgerichtete Rosettenblätter und die Blattfarbe ist graulich oder fast gelblich grün, ganz hell. Die Blattfarbe von *decurtatum* ist fast dunkel wie die von *tenebricans*, schwarzgrün. Die ausgewachsenen Rosetten haben kürzere Blätter als die der *pycnolobum*, weshalb sie kleiner erscheinen; auch sind sie stärker zerschlitzt. Diese Unterschiede gehen sehr gut aus den Fig. 1 und 2 hervor. Diese heben auch einen anderen, sehr charakteristischen Unterschied der Typen hervor, nämlich die kürzeren Stengel von *decurtatum*. Die äusseren Hüllblätter sind die festen, steif und kragenförmig abstehenden von *pycnolobum*, aber sie sind kürzer und der Kragen ist dichter. Durch die kurzen Stengel erinnert *decurtatum* an den *laeticolor*-Typus, bei dem sie jedoch so verkürzt sind, dass die Blütenköpfe von den Blättern fast überwachsen werden.

Dieser Typus erinnert wohl in bezug auf die Hüllblätter an *pycnolobum*, ist aber habituell ebenso stark von diesem abweichend wie z. B. *laeticolor*, *sublaeticolor* oder *laeticeps*. Der letztere Typus ist übrigens ausser in meinen Kulturen nur ein paarmal in der nächsten Umgebung von Lund aufgefunden worden (HAGLUND, 1934).

Unter den Typen gibt es also sowohl häufigere als auch seltenere. Aber der Unterschied ist in beiden Fällen ganz ausgeprägt. Unter den 51 Parzellen (Ausgangspflanzen) von *pycnolobum* oder den 29 von *sagittatum* waren ebensowenig feststellbare Unterschiede zu entdecken wie unter den 4 von *cordatum* oder den 2 von *xanthostigma*. Transgressionen traten nicht auf.

Die Sonderprägung der Typen war dagegen sehr verschieden und damit folgte die Leichtigkeit ihrer Unterscheidung. Die Typen *longisquameum* und *xanthostigma* erschienen mir so ähnlich, dass ich sie anfangs in eine Gruppe vereinigte. Erst als die Parzellen nebeneinander standen, traten auch ihre Unterschiede zutage. In ähnlicher Weise verhielten sich *brevisectum* und *mimulum*.

Überhaupt war eine Entscheidung der Frage, was ähnlich oder sehr abweichend war, zu grossem Teil davon abhängig, welche Merkmale für die Beurteilung ausgewählt wurden. Der *dilatatum*-Typus erschien mir, nachdem ich mein Material drei Generationen hindurch beobachtet

hatte, sowohl dem *longisquameum*- als dem *xanthostigma*-Typus sehr ähnlich. Sie schienen eine ganz intime Dreigruppe zu bilden. Betrachtete man aber die Sondermerkmale von *dilatatum*, so erinnerte der Typus durch seine stark zurückgeschlagenen Hüllblätter an *retroflexum*, in bezug auf gewisse Rosettenmerkmale an *cordatum* sowie in

Fig. 1. Der Mikrotypus *pycnolobum*.

bezug auf die Blütefarbe und die weitgeöffneten und lange offen stehenden Blütenköpfe an *pycnolobum*.

Das angeführte Beispiel von *dilatatum* könnte man für jeden Typus vervielfältigen. Sämtliche Typen bildeten offenbar *eine einzige Kombinationsgruppe* ein Kombinationsfeld, *keine Serie* oder Reihe, die in Sondergruppen aufgeteilt werden konnte.

*Dass von Taraxacum officinale auf einer ausgewählten Fläche eine*

*bestimmte und begrenzte Anzahl morphologisch distinkter Typen auftreten, die nicht durch kontinuierliche Zwischenformen verbunden sind, ist also durch meine Versuche festgestellt.*

Nun erscheint indessen die Frage berechtigt, ob nicht kleinere erbliche Formen vorkommen, nämlich solche, die *nicht morphologisch*

Fig. 2. Der Mikrotypus *decurtatum*. Die mit *pycnolobum* verglichen kleineren Rosetten und kürzeren Stengel treten deutlich zutage.

festgestellt werden können. Vielleicht gibt es physiologische Unterschiede, die die Assimilationsintensität, die Nährstoffsselektion, die Atmungskapazität usw. betreffen. In diesem Fall versagt das morphologische Experiment, mag es noch so sorgfältig durchgeführt sein, gänzlich. Der Einwand, dass es binnen den morphologischen Grundtypen kleinere Unterschiede geben könnten, wäre demnach nicht entkräftigt.

Dieser Einwand ist natürlich *theoretisch* berechtigt, aber kaum, wie

wir weiter sehen werden, real. Wir wissen nämlich nunmehr, dass die Wirkungsweise eines Gens pleiotrop ist. Ein Gen ist kein Organbildner, der eine einzige Eigenschaft bedingt, sondern das Gen schlägt im ganzen Organismus durch, und das nicht nur morphologisch, sondern auch biologisch und physiologisch. Das ist schon ein genischer Truismus.

Bei *Taraxacum* findet man, wenn eine Beobachtung der biologisch-physiologischen Eigenschaften der Typen vorgenommen wird, bald ganz ausgeprägte Unterschiede auch in dieser Hinsicht. Dies sei an einigen charakteristischen Beispielen veranschaulicht.

Gewisse Typen sind morphologisch dadurch ausgezeichnet, dass ihre Blattspreite nicht einheitlich grün ist, sondern einen braunen Blattsaum hat (*longisquameum*, *sublaeticolor*, *laeticeps*). Gleichzeitig zeigen sie stark verlängerte und helle Blattzipfel, helle Rosetten mit dem Boden stark anliegenden Blättern, in die Rosette tief versenkte junge Köpfchen und früheres Blühen im Frühjahr als die Mehrzahl der übrigen Typen. Mit dem Gen für das auffallendste morphologische Merkmal, das Makulieren, folgen also mehrere Eigenschaften der Struktur und Rhythmik der Pflanze.

Die Unterschiede in bezug auf das Erreichen der Blühfähigkeit während des ersten Entwicklungsjahres waren auch sehr ausgeprägt. Im Jahre 1934 wurden die Ende Mai geernteten Samen unmittelbar gesät und die jungen Pflanzen Anfang August ausgepflanzt. Ende Oktober blühten sämtliche Parzellen von *pycnolobum*, welcher Typus die grösste Anzahl von Nachkommen, nämlich 51, hatte. Von *sagittatum*, von dem ich die nächsthöchste Parzellenzahl hatte, nämlich 29, war bei keiner einzigen Nachkommenschaft eine blühende Pflanze zu sehen. Vereinzeltes Blühen zeigten *duplidens*, *polyodon* und *retroflexum*. Es ist also offenbar, dass gewisse physiologische Eigenschaften ebenso fest mit dem Typus verknüpft sind wie die morphologischen. Denn keine einzige *pycnolobum*-, wie auch keine einzige *sagittatum*-Nachkommenschaft, wich von ihrem Typus ab.

In bezug auf den Nyktitropismus der Blüten waren bei den verschiedenen Typen auch ganz deutliche Unterschiede festzustellen. Die Köpfchen sind bei ihrem »Öffnen» und »Schliessen» sehr von der Sonne abhängig. Diese Eigenschaft kann daher nur an Tagen mit voller Sonnenbestrahlung beurteilt werden. Eine Untersuchung, die unter optimalen Bedingungen vorgenommen wurde, zeigte, dass bei der Mehrzahl der Typen schon früh Nachmittags ein Schliessen der Köpfchen eintrat. Für *sagittatum* war diese Zeit schon ein wenig ausge-

dehnt, bis 2 Uhr, für *dilatatum* ganz markiert, bis 3 Uhr, für *pycnolobum* endlich sehr auffallend, bis 4 Uhr. Von besonderem Interesse ist, dass *pycnolobum*, welcher Typus in bezug auf die Blühperiode eine Verkürzung zeigt, in bezug auf die Periode des Nyktitropismus eine Verlängerung aufweist. Die ganze Rhythmik dieses Typus ist demnach sehr abweichend, wahrscheinlich bedingt durch ein Gen mit vielseitig physiologischem Effekt. Dass ein und dasselbe Gen diese verschiedenen und extremen Eigenschaften bedingt ist wahrscheinlich. Sie bezeichnen wohl nur zwei während der ontogenetischen Entfaltung der Pflanze aufeinander folgende Reaktionen desselben Agenz (Genwirkung). Und vielleicht ist dieses Gen dasselbe, das auch für viele der extremen morphologischen Merkmale des Typus verantwortlich ist.

Ein ganz und gar sonderartiger nyktitropischer Typus ist *laeticeps*. Während sämtliche andere Typen bei bewölktem Himmel ihre Köpfchen schliessen oder geschlossen halten, hat *laeticeps* auch unter diesen Verhältnissen weit geöffnete Köpfchen. Der Typus nimmt hierdurch eine ganz auffallende Sonderstellung im *Taraxacum*-Versuch ein.

Die physiologischen Eigenschaften der Typen zeigen eine ebenso grosse Übereinstimmung wie die morphologischen. Alle Linien eines Typus reagieren in bezug auf Tropismen und Periodizitäten offenbar ganz gleichartig. Wäre die Variabilität biologischer und physiologischer Eigenschaften grösser als die der taxonomischen und habituellen, also der morphologischen, so wäre auch zu erwarten, dass die Reaktionsweise in bezug auf die Lebensäusserungen der verschiedenen Linien eines Typus in einigen Fällen Unterschiede aufweisen sollten. Das traf nicht zu. Die physiologischen Merkmale sind daher, soweit die Untersuchung diesbezüglich durchgeführt worden ist, von ganz denselben Genen abhängig wie die morphologischen. Der Effekt dieser Gene ist nur stark pleiotrop.

Dieses Ergebnis macht es auch sehr wahrscheinlich, dass kleinere biologisch-physiologische Unterschiede, ebensowenig wie taxonomische, innerhalb der Typen vorhanden sind. Diese sind ganz gleichförmig. Wie sind sie dann vom genischen und variationsbiologischen Gesichtspunkt aufzufassen? Dies ergibt sich dann auch unmittelbar. Es ist nur eine Deutung möglich: Sie sind *die kleinsten erblichen Varianten* der Population *Taraxacum officinale. Sie sind die Genotypen* oder, falls man diesen ganz identischen Terminus bevorzügt, *sie sind die Biotypen.*

Es ist also höchst wahrscheinlich, dass die »Arten», die »Mikrospezies», die »Typen» — wie ich sie bis jetzt schlechthin genannt habe

— die von den Taxonomen bei *T. officinale* ausgeschieden und beschrieben worden sind, nur die Rekombinanten der Population darstellen, also die Biotypen. Ganz sicher kann man indessen diesbezüglich nicht sein. Der Biotypus ist ja ein *genischer* Begriff, der *absolute* Identität der Konstitution bedeutet. Es ist deshalb schwierig, ihn für systematische Zwecke zu verwenden. Denn auch wenn die Varianten, die »Mikrospezies», Biotypen sind, wie ich oben für *Taraxacum* wahrscheinlich machen konnte, so kann man es *nicht mit vollkommener Sicherheit* behaupten. Es besteht jedenfalls die *Möglichkeit*, dass zu ein und demselben Typus in seltenen Fällen mehr als ein Biotypus gehören kann, wenngleich die Unterschiede zu gering sind, um morphologisch oder physiologisch festgestellt werden zu können. Es ist unwahrscheinlich, aber möglich.

Es erscheint mir daher unrichtig, den Ausdruck Biotypus für systematische Einheiten zu verwenden, auch wenn man mit grösster Wahrscheinlichkeit annehmen kann, dass solche unterscheidbar sind. Aber ebenso unrichtig, ja widersinnig wäre es, eine Bezeichnung, die auf »Art» oder »Spezies» hinzielt, zu benutzen. Spezies bedeutet ja in der Systematik eine Population, einen Variationskreis, eine Kombinationssphäre, oft unzähliger Varianten. Eine »Mikrospezies» von *Taraxacum* ist ein *invariabler* Typus, ganz starr, *eine Kombination* innerhalb einer grösseren Population. Das zeigen die »Mikrospezies». Sie können nicht in Kleingruppen oder Reihen zusammengefasst werden, da ihre Gesamtheit eine Population bildet, und sie selbst stellen nur die verschiedenen Kombinationen gewisser Grundgene dar.

Aber sie sind konstant, sagt man; sie behalten ihre Eigenschaften auch in Kulturversuchen unverändert bei. Natürlich, aber nur weil sie agamosperm sind. Sie würden aber, falls Samenvermehrung eintreten könnte, keine Konstanz aufweisen. Denn die Mehrzahl der Typen sind laut den Untersuchungen von GUSTAFSSON (1932) triploid. In der Nachkommenschaft *muss* dann Variabilität, und zwar eine sehr reiche und auffällige, eintreten. Das zeigen ja die fast unzähligen während den letzten Jahrzehnten ausgeführten Untersuchungen von Triploiden verschiedener Gattungen zu voller Evidenz. Da aber keine Befruchtung stattfindet, sondern die Vermehrung ganz vegetativ vor sich geht, wird ja die Reproduktion rein klonal, ganz als ob sie z. B. durch abfallende Bulbillen verursacht würde. Die »Konstanz» der Typen kann daher als Begründung für eine Speziesbezeichnung gar nicht herangezogen werden. Denn solchenfalls müssten alle Klonen des Pflanzenreiches als

Spezies aufgefasst werden. Und das ist eine unheimliche Konsequenz, vor der *jeder* Systematiker zurückschrecken würde.

Da man im abgehandelten Fall keinesfalls die Bezeichnung Spezies und nicht gern den Ausdruck Biotypus für die Varianten verwenden kann, erscheint es mir notwendig, sie mit einem neuen Ausdruck zu belegen. Ein solcher, der mir sehr adäquat erscheint, ist *Mikrotypus.* Dieser besagt, dass wir es mit den *kleinsten unterscheidbaren* Varianten zu tun haben, und nicht mehr. Er hebt ausserdem durch den »Typus«-Begriff hervor, dass diese keine kontinuierliche, untrennbare Reihe bilden, sondern als *habituell* gut umschriebene Varietäten je für sich gekennzeichnet werden können. *Eine grössere Population, Taraxacum officinale, kann also bei genauem Durchforschen durch Spezialisten restlos in ihre Mikrotypen aufgelöst werden.*

Aber weshalb, kann man nun einwenden, ist es bei *Taraxacum* möglich, Mikrotypen zu unterscheiden? Ist es nicht paradoxal, dass man bei einer Spezies, die eine ausserordentliche Variabilität aufweist, eine ungeheure Anzahl von Varianten unterscheiden kann, während man bei der Mehrzahl der Arten des Pflanzenreiches nur eine fast kontinuierliche Variation beobachtet, und in diesem Falle die wenigen, markierteren Varianten gar nicht zu bestimmten Typen zusammenfassen kann?

Die Erklärung dieser früher ganz unverständlichen Tatsache ist indessen gewiss auch in der besonderen Vermehrungsweise bei *Taraxacum* zu suchen. Sie ist nur die Folge der agamospermen Samenbildung.

Bei einer agamospermen Pflanzenart muss die Selektion eine sehr viel effektivere Wirkung haben als bei einem sexuellen Fremdbefruchter. Bei letzterem ist die Kombination frei; die Biotypen müssen ganz kaleidoskopisch entstehen und verschwinden. Die schwächeren Biotypen werden wohl ausgemerzt, aber sie werden durch die Gameten anderer Pflanzen der Population wieder neugebildet. Ihr Gametentypus »parasitiert« an den vitalen Pflanzen. Die Variabilität wird sehr zähe in ihrem ganzen Umfang festgehalten. Nur sinkt natürlich die Frequenz der avitalen Typen. *Vollkommen* ausgemerzt werden sie, wie eine theoretische Überlegung zeigt, niemals.

Eine ganz andere Wirkung hat dagegen die Selektion an einer Spezies, die agamosperme Fortpflanzung erhalten hat. Die Agamospermie ist bei den Kompositeen, wie schon MENDEL (1869) für *Hieracium* gezeigt hat und wie OSTENFELD (1910) später bestätigen konnte, dominant. Wird also ein teilweise agamospermer Biotypus mit einem sexuellen gekreuzt, so wird die ganze Nachkommenschaft agamo-

sperm. Hieraus folgt, dass die agamospermen Biotypen in der Population (Spezies) mehr und mehr zunehmen müssen, bis endlich ihr ganzer Biotypeninhalt von der Agamospermie »fixiert» worden ist. Alle Biotypen sind dann »konstant» und geben nur ganz identische Nachkommen; sie sind zu Klonen geworden.

Nun setzt die Selektion mit einer gewaltigen Wirkung ein. Denn alles was nicht vital ist wird *definitiv* ausgemerzt. Alle schwachen Biotypen verschwinden. Ihre Neubildung ist ausgeschlossen, denn es findet kein sexueller Prozess mehr statt. Nach und nach gehen neue Biotypen zu Grunde, die nicht ganz konkurrenzkräftig sind. Die Spezies, die ursprünglich als sexuell vielleicht Millionen von verschiedenen Biotypen gehabt hat — diese Anzahl wird schon durch die Kombination von 20 Genen gebildet — verarmt allmählich an Biotypen. Diese Verarmung schreitet schnell vorwärts, reduziert die Million auf Hunderttausend, Hunderttausend auf Tausend und Tausend auf einige Hundert.

Und was geschieht dann? Ja, die *verbleibenden* Biotypen beginnen *morphologisch sichtbar* zu werden. *Die Spezies ist polymorph geworden.* Aber weshalb? Weil ihre ursprüngliche, sehr *grosse* Variabilität *ausserordentlich* stark *herabgesetzt* worden ist. Dies ist der wirkliche Gang der Erscheinung der Polymorphie! Die Polymorphie beruht nicht auf einer Vermehrung der Varianten, sondern auf einer ganz katastrophalen Verminderung ihrer Anzahl.

Was ist es also, das in einer polymorphen Art übrig bleibt? Die Biotypen. Ganz wie früher nur die Biotypen. Aber nur gewisse und sehr wenige Biotypen. Die vitalen Biotypen. Nun sehen wir klar, wie unsinnig es ist, diese dann Spezies zu nennen.

Wahrscheinlich bezeichnen die 450 unterschiedenen skandinavischen *Taraxaca* den ganzen oder sehr nahe den ganzen Biotypeninhalt der alten Spezies *T. officinale.* Nur weil es aus den oben angeführten Gründen nicht als zulässig angesehen werden kann, den nur in streng konstitutioneller Bedeutung verwendbaren Ausdruck Biotypus als morphologische Bezeichnung zu benutzen, wählen wir für diese wahrscheinlichen Biotypen den Ausdruck Mikrotypen. Mit dem sorgfältigen Feststellen der *Mikrotypen* einer *vollständig agamospermen Spezies* haben wir auch, jedenfalls sehr nahe, ihren *Biotypeninhalt* ermittelt.

Wir haben also nunmehr einen ganz klaren Einblick in die Ursachen der Polymorphie gewisser Arten und Gattungen. Wir wissen, dass ihre Komponenten nichts anders als Restbiotypen sind. In diesem Punkt ist also eine weitere Erörterung, die ihre »Rangordnung» feststellen sollte, ganz überflüssig. Sie sind die *kleinsten* Bausteine der

Spezies. Sie haben keinen Rang. Ebensowenig wie die Ziegelsteine eines Hauses vor dem Bauen einen Rang von differenzierten Teilen des Gebäudes haben.

Es verbleibt nun die Frage, ob die Population *Taraxacum officinale* als eine einzige Spezies, *T. officinale* WEB., aufgefasst oder ob diese in weitere Formenkreise, die pflanzengeographisch, ökologisch und zytologisch mehr mit den Artpopulationen allogamer Spezies übereinstimmen, zergliedert werden soll. Ich möchte hier nicht näher auf diese Frage eingehen — ich werde sie im Zusammenhang mit anderen »kritischen» Genera in einer grösseren Arbeit eingehender behandeln — sondern nur andeuten, dass ich im allgemeinen die Subgenera DAHLSTEDTs als Spezies betrachte (also *T. vulgare*, *palustre*, *ceratophorum* etc.). Eine zytologisch-ökologische Begründung hierfür hat GUSTAFSSON (1935) veröffentlicht. Eine genisch-experimentelle ist wegen der Agamospermie ganz ausgeschlossen.

Eine Aufteilung einer Spezies in ihre Mikrotypen ist natürlich nur bei agamospermen oder überhaupt bei apomiktischen Arten durchführbar. Schon die amphimiktischen Autogamen sind gewöhnlich keiner so stark selektiven Elimination von Biotypen ausgesetzt wie die Agamospermen, weil Kreuzungen zufällig vorkommen, durch die dann neue Biotypenschwärme geschaffen werden. Dass jedoch, wenn Vizinismus selten stattfindet, Variabilitätsverhältnisse auftreten, die sehr auffallend an die von *Taraxacum* erinnern, das zeigen die seit JORDAN bekannten mikrotypenähnlichen Erscheinungen bei *Erophila verna* (L.) F. CHEV. Das Zerfallen einer *vollständig* autogamen Spezies in Mikrotypen ist eine ebenso absolute Notwendigkeit wie das einer *vollständig* agamospermen. Die genische und selektive Konsequenz führt in beiden Fällen auf verschiedenen befruchtungsbiologischen Wegen zum selben Ziel (HERIBERT NILSSON, 1930).

Die selektive Verarmung des Biotypeninhalts von *Taraxacum*, wie sie oben dargestellt worden ist, ist natürlich nur unter der Voraussetzung gültig, dass keine Neubildung von Mikrotypen stattfindet.

In bezug auf diese Frage scheinen die Taraxakologen durch ihre Feldstudien zu der Auffassung gekommen zu sein, dass die Mikrotypenzahl begrenzt ist und dass neue Typen nicht auftreten. Vor allem hat MARKLUND (1940) in einer umfassenden Arbeit diese Ansicht bestimmt vertreten. »Unter den Arten», hebt er hervor, »die in Nyland sicher oder möglicherweise in natürlichen Pflanzenvereinen heimisch sind, gibt es, wie aus dieser Darstellung hervorgegangen ist, gar keine im Untersuchungsgebiet oder überhaupt in Finnland endemischen. Nichts

deutet somit darauf hin, dass die in natürlicher Vegetation gedeihende *Taraxacum*-Flora des untersuchten Gebietes während der postglazialen Zeit den aller geringsten Zuschuss durch Neuentstehung erhalten hätte» (l. c., p. 36). Nur an ausgeprägten Kulturstandorten findet er eine nicht unbeträchtliche Anzahl von Mikrotypen, die nirgendwo ausserhalb des Gebietes gefunden worden sind. Schon ihr Auftreten betrachtet er als sehr bezeichnend dafür, dass sie eingeschleppt worden sind.

Das Resultat der Feldstudien von MARKLUND scheint also die Auffassung zu bestätigen, dass die selektive Verarmung an Biotypen nicht durch eine Neubildung von solchen kompensiert wird. Die Depauperierung der Population an Varianten ist endgültig, ganz wie man dies auf Grund von genischen Überlegungen zu erwarten hat.

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Bei Abschluss meines Manuskripts erhielt ich die sehr schöne experimentelle Arbeit von SØRENSEN und GUDJÓNSSON (1946) über *Taraxacum*. Sie führen ganz unwidersprechlich den Beweis durch, dass auch jetzt neue Mikrotypen entstehen. Vor allem weisen sie das Auftreten von Chromosomenvarianten, Aberranten, nach, wie sie diese mit einem von WINGE eingeführten Ausdruck nennen.

Diese Aberranten entstehen durch das Wegfallen oder Hinzukommen eines, in gewissen Fällen mehrerer Chromosomen. Da die diploide Zahl 24 ist, haben die neuen Formen $24-1$, $24-2$, $24-3$, $24-4$, $24-5$, $24+1$, $24+2$. Ausserdem wurden Polyploide gefunden, die sowohl Verdoppelung der Grundzahl (also $2n=48$) wie aneuploide Verdoppelung $2n=2(24-1)=46$ usw. zeigten. Hierdurch erhält man eine sehr ergiebige Quelle für die Produktion neuer Formen.

Die erwähnten Forscher haben auch, besonders für die Zahl $2n=23$, bewiesen, dass wirklich alle erwarteten Aberranten auftreten. Da die Grundzahl $n=8$ ist — was GUDJÓNSSON durch das Auftreten von 3 ganz ähnlichen Gruppen von Chromosomen im Karyotypus zytologisch festgestellt hat — so sind 8 verschiedene Aberranten zu erwarten. Sie sind auch alle gefunden und beschrieben worden.

Was ist nun die weitere Konsequenz dieser chromosomalen Verhältnisse? Natürlich die, dass *sämtliche* diese vielen Aberranten bei *allen* Mikrotypen vorkommen können. Mit anderen Worten: Jeder Mikrotypus kann sich mit jeder Aberrante kombinieren. Von Mikrotypus *pycnolobum* hat man z. B. 8 Aberranten. Aber auch bei den übrigen konstatierten, sagen wir 449, Mikrotypen sind von jedem 8 neue

Formen zu erwarten. Das macht schon 3.592. Aber dazu kommen neue Aberranten von den neuen aneuploiden Zahlen (24 — 2, usw.) und neue Polyploide und aneuploide Polyploide. Die mögliche Anzahl neuer Aberranten steigt damit fast in das Unermessliche.

Diese Resultate sind experimentell begründet. Dass also eine *überaus reiche Neubildung* von Biotypen innerhalb der Mikrotypen stattfindet ist *sicher.*

Wie lassen sich nun diese Resultate mit den ganz entgegengesetzten der Taxonomen vereinigen? Und weiter fragt man: Weshalb haben die Taraxakologen, die oft Kulturversuche ausgeführt haben, nichts von dieser Erscheinung beobachtet?

Eine gewiss richtige Erklärung für die letztgenannte Diskrepanz geben SØRENSEN und GUDJÓNSSON in ihrer Arbeit (p. 7): teils hat man nur winzige Nachkommenschaften aufgezogen, teils sind beim Auspflanzen gewiss die kräftigeren Pflanzen ganz unbewusst ausgelesen worden. Meine Methodik kränkelte gewiss an diesen beiden Fehlerquellen. Aber meine Versuche bezweckten nicht *neue* Varianten aufzusuchen, sondern ich wollte nur die *vorhandene* Variabilität feststellen. Als Untersuchungen über eine Formenneubildung haben sie deshalb keinen Wert.

Von den SØRENSENschen Aberranten habe ich nur eine einzige gefunden. In einer Nachkommenschaft von *polyodon* trat 1934 eine abweichende, diffus gelbblättrige Pflanze auf. Wie ich jetzt sehe, stimmt sie sehr gut mit der Aberrante *olivacea* überein. Diese trat auch in den Versuchen von SØRENSEN gerade bei *polyodon* »fairly frequently» auf.

Noch eine abweichende Form, die mit keiner seiner erwähnten Aberranten übereinstimmt, trat in einer Nachkommenschaft von *fulvum* auf. Die Pflanze wich von ihrem Mikrotypus durch ihren sehr üppigen Wuchs ab. Das Format war in bezug auf alle Organe das doppelte. Fig. 3 zeigt dies sehr gut. Rechts sieht man eine Parzelle des *fulvum*-Mikrotypus, links eine ihres Abweichers. Der Unterschied ist auffallend.

Durch das Aussehen des Abweichers war ich sogleich fest davon überzeugt, dass hier eine polyploide Form vorlag. Leider wurde keine Chromosomenbestimmung ausgeführt. Meine Versuche wurden 1939 abgebrochen, weil ich das mir gesetzte Ziel damals als erreicht betrachtete. Als ich 1945 die Versuche mit *fulvum* und ihrer abweichenden Form wiederaufnehmen wollte, zeigte es sich, dass die Samen nicht mehr keimfähig waren.

Die Resultate von SØRENSEN zeigen nun, dass die Polyploide gar

Fig. 3. Der Mikrolypus *fulvum* (rechts) und sein *giganteum*-Abweicher (links).

nicht das Aussehen meines *giganteum*-Typus haben. Sie sind kleiner als ihre Muttermikrotypen, sie haben einen gedrungenen Wuchs. Die Blütenköpfchen sind indessen auffallend gross. Die Pflanze ist deshalb sehr disproportioniert. Von einem Gesamtriesen wie bei meinem *giganteum*-Abweicher ist dort keine Rede. Dieser muss daher eine Neuvariante ganz anderer Natur darstellen als der einer Tetraploide (Hexaploide). Leider ist nun nicht einmal das Ermitteln ihrer Chromosomenzahl mehr möglich.

Dass der *giganteum*-Abweicher aus *fulvum* hervorgegangen ist, darüber besteht kein Zweifel. Er hat nicht nur den Habitus dieses Mikrotypus, nur im grösseren Format, beibehalten. Er hat auch die sehr charakteristische Samenfarbe dieses Typus, die nicht ziegelrot, wie bei der Mehrzahl der übrigen *Erythrosperma*, sondern hell gelbrot (terrakotta) ist. Ferner ist keine ähnliche Form unter den schwedischen *Taraxaca* bekannt.

Durch die Untersuchung von SØRENSEN und GUDJÓNSSON ist also festgestellt, dass bei *Taraxacum* sehr häufig chromosomale Varianten auftreten. Für die beiden am ausführlichsten untersuchten Mikrotypen, *lacinosifrons* und *polyodon*, geben sie den Prozentsatz 2,1 resp. 1,3 an. Aber einzelne Mikrotypen zeigen eine noch stärker erhöhte Aberrantenproduktion. Für *protractifrons* finden sie 8 %, für *expallidiforme* 4,2 %. Bei anderen Mikrotypen war der Prozentsatz dagegen viel niedriger; bei *bracteatum* 0,2 % und bei *copidophyllum* 0,1 %.

Diese *überaus reiche Produktion* von Aberranten, also auch von *neuen Mikrotypen*, scheint meine oben angeführte Ansicht über den Effekt der selektiven Verarmung bei *Taraxacum* umzustürzen. Denn man muss wohl annehmen, dass die Selektion durch diese reiche Mikrotypenneubildung kompensiert werden sollte.

Das ist aber keineswegs sicher. Wie ich sogleich zeigen werde, ist es ganz unwahrscheinlich.

Studiert man die Angaben von SØRENSEN über die Vitalität der Aberranten, so findet man, dass sie schwächer sind als ihre Muttermikrotypen. So ist z. B. bei *elegans* »the fertility noticeably reduced, the heads always containing a relatively large number of empty achenes». Über *truncata* heisst es: »This aberrant shows some tendency to collapse during the flowering, giving rise to 'gaps' in the rows»; und weiter: »The fertility is somewhat reduced, a great many empty or poorly developed achenes being always present». Über *hamosa* wird berichtet, dass »after the flowering and ripening of the fruits in the second or third year a larger or smaller number of the plants will die

without any previous symptoms of weakness». Die Aberrante *olivacea* hat, wie oben erwähnt, diffus gelbgefleckte Blätter. Sie hat zarteren Wuchs und kleinere Köpfchen als ihr Muttermikrotypus, was wohl mit einer herabgesetzten Assimilation der gelblichen Blätter einhergeht. Die *crassifolia* bildet keineswegs eine kräftige Pflanze. Sie ist kleinwüchsig mit sehr kleinen Köpfchen, die ein auffallend grosses Involucrum haben. Ihre Blätter sind klein, aber sehr dick. *Tenuis* bildet eine kleine, fast sterile Pflanze und *pygmaea* ist ein Zwerg mit einem Wurzelsystem wie ein Hexenbesen.

Alle diese sieben Aberranten haben offenbare konstitutionelle Schwächen, die ihre Vitalität stark herabsetzen und ihre Konkurrenzkraft auf ein Minimum reduzieren.

Nur eine der primären Aberranten (2n — 1) ist kräftig und scheint in bezug auf Bau und Fertilität ihrem Muttermikrotypus gleichwertig zu sein, nämlich *plumosa*. Aber auch diese zeigt Eigenschaften, die auf eine disharmonische Konstitution hinweisen. Die Blätter sind z. B. konvex hinübergebogen, etwas gekräuselt, was eine nicht ungewöhnliche Erscheinung bei Mutanten mit herabgesetzter Vitalität (z. B. bei *Oenothera*) ist.

Dass sämtliche diese Aberranten eine geringe Konkurrenzkraft haben, geht auch aus der Tatsache hervor, dass sie von SØRENSEN nur einige wenige Male *in der Natur* gefunden worden sind. Sie sind dort also *sehr selten*. *Truncata* hat er dort nur viermal angetroffen, *elegans* zweimal, *plumosa* einmal. Das ist alles.

Dass die Aberranten von SØRENSEN eine so geringe Konkurrenzkraft besitzen, dass ihr Selektionswert auf Null sinkt, wird dadurch bestätigt, dass sie den Taraxakologen *aus der Natur unbekannt* sind. Skandinavische *Taraxacum*-Forscher, die sich lebenslang mit der Unterscheidung der kleinsten Varianten in der Natur beschäftigt haben und die ein ausserordentlich geübtes Auge für neuauftauchende Formen besitzen, kennen die *grosse* und *auffällige* Variabilität in den Kulturen von SØRENSEN nicht. Hierfür gibt es nur eine einzige Erklärung. Sie sind dort nicht zu finden. Sie werden nach ihrer Entstehung sehr schnell wieder ausgemerzt.

Natürlich können die Aberranten in der Natur ebenso *entstehen* wie im Versuchsgarten von SØRENSEN. Es erscheint wohl ausgeschlossen, dass das Versetzen von *Taraxaca* in einen Garten eine Mutabilität auslösen würde. *Taraxacum* ist ja schon stark kulturgebunden, jedenfalls die *Vulgaria*-Formen. Zufällig kann man sie in der Natur antreffen, aber sehr selten, wie ich oben berichtet habe. SØRENSEN

erwähnt auch (l. c., p. 15), dass LINDBERG FIL. ein einzigesmal seine Aberrante *plumosa* gefunden und sie auch kultiviert hat. Das zeigt ja, dass das Unterscheiden der Aberranten den Taraxakologen keine Schwierigkeit bereitet. Aber es kommt nur ganz zufällig vor, dass sie eine solche Pflanze zu sehen bekommen, weil die Aberranten sich nur ganz ohne Konkurrenz durchsetzen können. An zufällig vegetationsfreien Flecken können ihre Samen keimen und sich zu Pflanzen entwickeln. Sobald ein konkurrierender Bestand aufwächst, verschwinden sie.

Aus den angeführten Tatsachen ergibt sich, dass die grosse und auffallende Produktion von neuen Mikrotypen, die durch die Versuche von SØRENSEN und GUDJÓNSSON nachgewiesen worden ist, meine Schlüsse in bezug auf die natürliche Variabilität, den Biotypeninhalt und die selektive Verarmung bei *Taraxacum* ganz unberührt lassen. Falls ihre Ergebnisse meine Schlussfolgerungen beeinflussen, so ist es in positiver Richtung.

Man muss nämlich sagen, dass die neuen Tatsachen die Auffassung des selektiven Ausmerzens grosser Mengen von Biotypen beweisen. Sie beweisen nämlich, dass auch ganze Schwärme von Mikrotypen, von deren Existenz wir bis jetzt nichts wussten, *auftreten* und *verschwinden* wie ein Rauch im Sturm. In ähnlicher Weise verschwand auch die Mehrzahl der rekombinierten Biotypen, nämlich alle nicht vollvitalen, sobald die Variabilität durch die Agamospermie fixiert worden war. Sie waren durch das rekombinierende Spiel nicht mehr zu retten. Und die jetzt noch entstehenden neuen Mikrotypen sind schon vom Anfang an dem selektiven Ausmerzen preisgegeben. Sie sind wohl übrigens auch nicht neu, sondern genische Neukombinationen, die durch die Konstitution ihrer Spezies ursächlich und gesetzmässig bedingt sind. Sie erscheinen deshalb immer wieder von neuem. Und sie sind gewiss ebenso alt wie die Spezies.

Die oben angeführten Tatsachen und Schlussfolgerungen dürften wohl zeigen, dass zur Lösung des Problems der polymorphen Spezies in keinem Falle besondere Variabilitätserscheinungen angenommen werden brauchen. Die Ursache der grossen Anzahl unterscheidbarer Varianten beruht nämlich nicht, wie früher angenommen wurde, auf einer reichen Neubildung vitaler Varianten, sondern im Gegenteil auf einer ausserordentlich scharf selektiven Elimination von Biotypen der Speziespopulation. Die Restbiotypen treten dann, weil sie von Millionen bis auf Hunderte reduziert worden sind, für das Auge — jedenfalls der Spezialisten unter den Taxonomen — zutage. Was sie aber als Neu-

heiten beobachten, und was viele Artbildungstheoretiker mit Enthusiasmus als Zeichen einer Artbildung im Fluss auffassen, ist uralt.

## SUMMARY.

Title of paper: *Total inventorization of micro-types on a minimum area of Taraxacum officinale.*

The taxonomists have distinguished about 450 so-called »micro-species» of *Taraxacum officinale* WEB. in Scandinavia. A natural question is then whether the variation is exhausted with this, or whether these micro-species are collective or bridging forms exist between them.

For the purpose of solving this question the author collected from a limited area, only 50 square metres, seeds from *all* the *Taraxacum* plants growing there. These consisted of 177. On cultivation in plots followed by repeated seed-gathering and cultivation for five years it was found that 20 distinct types occurred on this minimum area, 19 of which represented previously described »micro-species» and only one was new. There was *no* variation round or between these types, the whole variation being exhausted with these distinct types. Of e. g. the *pycnolobum* »micro-species», which was represented by 51 initial plants, all plots in the progeny were perfectly alike. At the repeated tests undertaken not the slightest variation could be discovered between the 51 plots or between the plants within a plot.

The morphological conformity was accompanied by a physiological one. Within one »micro-species» there was no variation as regards rhythm of development, flowering time or nyctitropism, but an extremely marked such between different »micro-species».

It is therefore probable that »micro-species» are to be regarded as biotypes, distinct biotypes, between which there are no bridging forms. The number of biotypes in the Scandinavian population of *Taraxacum officinale* WEB. would thus not seem to be more than about 500. Just because the biotype number is so small specialized taxonomists are able to distinguish them.

As »micro-species» are biotypes, it is impossible to regard them as species. They are the least conceivable variants in a species. Naturally they cannot then be species themselves. They are constant, but this is on account of their agamospermous reproduction. They are clones, and if we were to call every such in the vegetable kingdom a species we should have to calculate these in at least billions, i. e. in astronomical figures.

Highly probably the distinguished morphological types are really biotypes. This genetic term, however, is hardly serviceable in taxonomy. For it means absolute constitutional identity. As we cannot declare with full certainty on the basis of ocular judgment that absolutely identical individuals are always brought together under our name of a type, it is necessary to create a new term for the least *distinguishable* units. I suggest the term *micro-type* for such. Judging from my results, however, it seems an indubitable fact that the micro-types in *Taraxacum* are biotypes.

*T. officinale* is a polymorphic species, and such a species has always been considered to be extremely variable. But this is not the case. Instead, the number of micro-types is strikingly small. Even at only 20 character differences over 1.000.000 biotypes are formed in a cross-fertilizer. About 500 in *Taraxacum* is an extremely small number.

That the number of micro-types has fallen so much in *Taraxacum* is an inevitable consequence of its agamospermous propagation. All micro-types that are not fully vital are eliminated through selection, while in a cross-fertilizer they persist in a certain percentage on account of panmixia. In an agamospermous species swarms of biotypes will be *entirely* obliterated, and hence the number must successively fall until only the fully vital are left. These then appear as distinguishable, i. e. as micro-types, as a consequence of the selective breakdown of the former continuous chain of variation.

However paradoxically it may seem, a polymorphic species will therefore be critical on account of its low variability. Polymorphy is by no means caused by the fact that a lively formation of species goes on, as is usually held, but by a mass death.

The rich production of chromosomally different forms out of *Taraxacum* micro-types found by SØRENSEN and GUDJÓNSSON does not affect the results and conclusions presented here, as these forms are not vital and are not to be found in nature except by pure chance.

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## ZITIERTE LITERATUR.

1. GUSTAFSSON, Å. 1932. Zytologische und experimentelle Studien in der Gattung *Taraxacum*. — Hereditas XVI.
2. — 1935. The importance of the apomicts for plant-geography. — Bot. Notiser 1935.
3. HAGLUND, G. 1934. Några nya eller i Sverige nyfunna *Taraxacum*-arter. — Bot. Notiser 1934.

4. HYLANDER, N. 1941. Förteckning över Skandinaviens växter. 1. Kärlväxter. — Lund.
5. MARKLUND, G. 1940. Die *Taraxacum*-Flora Nylands. — Acta botanica fennica, 26.
6. MENDEL, G. 1869. Ueber einige aus künstlicher Befruchtung gewonnene *Hieracium*bastarde. — Verh. naturforsch. Ver. Brünn, 8.
7. NILSSON, HERIBERT. 1930. Synthetische Bastardierungsversuche in der Gattung *Salix*. — Lunds Univ. Årsskrift, N. F. 27.
8. OSTENFELD, C. H. 1910. Further studies on the apogamy and hybridization of the *Hieracia*. — Zschr. f. ind. Abst. u. Vererb. 3.
9. SØRENSEN, TH. and GUDJÓNSSON, G. 1946. Spontaneous chromosome-aberrants in apomictic *Taraxaca*. — Kgl. Danske Vidensk. Selskab, Biol. Skrifter 4, Nr. 2.

# THE GENETIC EFFECTS OF BREEDING IN SMALL POPULATIONS

## A DEMONSTRATION FOR USE IN GENETIC TEACHING

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WHEN teaching Elementary Science it is always important to arrange the demonstrations and the experiments in such a way that the essentials are brought out as obviously as possible. But the obstacles may be numerous. For instance in Genetics, when the question is to demonstrate the homozygotizing effect of inbreeding in allogamous species, the difficulties are very great if it is desired to use living material: the necessarily great number of generations are impossible to come up to during the limited space of time, absolute fertility equality of the different animals is practically never fulfilled, and really free intercrossings are very difficult to ensure. And even if all these practically unsurmountable difficulties could be overcome, the effect of dominance (and of epistasis in the case of two or more pairs of genes) would still make it impossible to determine exactly the number of generations after which all heterozygosity has been eliminated.

As it must nevertheless be looked upon as very important in Genetics class work to demonstrate the principal effects of inbreeding, other ways must be sought. To give WRIGHT's mathematical deductions (1921 a, 1931) would, however, usually be far above the horizon of the auditors. And if the demonstration is confined to the exposition of his well-known curves (1921 b, 1931), the most important part, viz. the effect of random causes, would be difficult to make clear. In my Genetics class, therefore, I have for a number of years made use of a kind of roulette-game. Of course, any other machinery than a roulette may likewise be used if only all the necessary probabilities can be covered. A table of random numbers may also be useful. This roulette system has been very successful, and as it may be of some value for other teachers in Genetics it will be demonstrated here.

The demonstrations have been confined to the case of 1 pair of genes, *A* and *a*. Instead of free intercrossing it is thought that every generation is propagated by a limited number of monogamous parental pairs. The principal results will be the same in both cases, and it is simpler to work with pairs of monogamous parents than with free intercrossing. Suppose, thus, that in each generation there are $n$ pairs of parents and that of these, in a certain generation, there are

$n_1$ pairs $AA \times AA$
$n_2$ » $AA \times Aa$
$n_3$ » $AA \times aa$
$n_4$ » $Aa \times Aa$
$n_5$ » $Aa \times aa$
$n_6$ » $aa \times aa$

By working out the kind of progeny from each type of parental combination it will immediately be found that the proportions of the three genotypes *AA*, *Aa*, and *aa* in the next generation are

$$\frac{4n_1 + 2n_2 + n_4}{4n}, \quad \frac{2n_2 + 4n_3 + 2n_4 + 2n_5}{4n}, \quad \frac{n_4 + 2n_5 + 4n_6}{4n} \qquad (1)$$

These three ratios thus also give the probabilities that a parent within the next generation will belong to one or other of the three possible genotypes. And these probabilities are — at least for the smallest values of $n$ — easy to realize with the aid of a roulette.

In the Genetics class the students usually have to work out the cases of $n = 1$, 2, and 5. They are therefore given sheets containing working tables. Tables 1 and 2 show these working tables for $n = 1$

TABLE 1. *Working table for $n = 1$ (brother—sister matings).*

| Parental generation | | | | | | Progeny generation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Probability in fourths of | | | Roulette | | |
| $n_1$ | $n_2$ | $n_3$ | $n_4$ | $n_5$ | $n_6$ | *AA* | *Aa* | *aa* | *AA* | *Aa* | *aa* |
| 1 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | all | — | — |
| 0 | 1 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 1—18 | 19—36 | — |
| 0 | 0 | 1 | 0 | 0 | 0 | 0 | 4 | 0 | — | all | — |
| 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 1 | 28—36 | 10—27 | 1—9 |
| 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 2 | — | 1—18 | 19—36 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 4 | — | — | all |

TABLE 2. *Working table for* $n = 2$.

| Parental generation | | | | | | Progeny generation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Probability in eighths of | | | Roulette | | |
| $n_1$ | $n_2$ | $n_3$ | $n_4$ | $n_5$ | $n_6$ | *AA* | *Aa* | *aa* | *AA* | *Aa* | *aa* |
| 2 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | all | — | — |
| 1 | 1 | 0 | 0 | 0 | 0 | 6 | 2 | 0 | 1—27 | 28—36 | — |
| 1 | 0 | 1 | 0 | 0 | 0 | 4 | 4 | 0 | 19—36 | 1—18 | — |
| 1 | 0 | 0 | 1 | 0 | 0 | 5 | 2 | 1 | 1—20 | 21—28 | 29—32 |
| 1 | 0 | 0 | 0 | 1 | 0 | 4 | 2 | 2 | 19—36 | 10—18 | 1—9 |
| 1 | 0 | 0 | 0 | 0 | 1 | 4 | 0 | 4 | 1—18 | — | 19—36 |
| 0 | 2 | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 19—36 | 1—18 | — |
| 0 | 1 | 1 | 0 | 0 | 0 | 2 | 6 | 0 | 1—9 | 10—36 | — |
| 0 | 1 | 0 | 1 | 0 | 0 | 3 | 4 | 1 | 21—32 | 5—20 | 1—4 |
| 0 | 1 | 0 | 0 | 1 | 0 | 2 | 4 | 2 | 1—9 | 10—27 | 28—36 |
| 0 | 1 | 0 | 0 | 0 | 1 | 2 | 2 | 4 | 28—36 | 19—27 | 1—18 |
| 0 | 0 | 2 | 0 | 0 | 0 | 0 | 8 | 0 | — | all | — |
| 0 | 0 | 1 | 1 | 0 | 0 | 1 | 6 | 1 | 1—4 | 5—28 | 29—32 |
| 0 | 0 | 1 | 0 | 1 | 0 | 0 | 6 | 2 | — | 10—36 | 1—9 |
| 0 | 0 | 1 | 0 | 0 | 1 | 0 | 4 | 4 | — | 1—18 | 19—36 |
| 0 | 0 | 0 | 2 | 0 | 0 | 2 | 4 | 2 | 28—36 | 10—27 | 1—9 |
| 0 | 0 | 0 | 1 | 1 | 0 | 1 | 4 | 3 | 1—4 | 5—20 | 21—32 |
| 0 | 0 | 0 | 1 | 0 | 1 | 1 | 2 | 5 | 29—32 | 21—28 | 1—20 |
| 0 | 0 | 0 | 0 | 2 | 0 | 0 | 4 | 4 | — | 1—18 | 19—36 |
| 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 6 | — | 28—36 | 1—27 |
| 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 8 | — | — | all |

and 2, which are constructed so as to make the number of holes in the roulette, corresponding to the three genotypes in the offspring generation, to be in the proportions (1). As it is possible to combine the integers $n_1$, $n_2$, $n_3$, $n_4$, $n_5$, $n_6$, the sum of which is $n$, in $\frac{(n+5)}{n!\,5!}$ different ways, the number of entries for $n = 1$ (brother—sister matings, Table 1) is 6, and for $n = 2$ (Table 2) the number of entries is 21. The students who work with 5 pairs of parents get a working table with $\frac{10!}{5!\ 5!} = 252$ entries (not shown here).

When the experiment starts it is thought that the population consists of only heterozygous individuals, i. e. it is a $F_1$ population. Therefore $n_4 = n$ and $n_1 = n_2 = n_3 = n_5 = n_6 = 0$. Suppose now we are dealing with the case of *2 pairs* of parents per generation. From the working table (Table 2) the student finds that in the progeny ($F_2$)

after $n_4 = 2$ the numbers of *AA*, *Aa*, and *aa* individuals are (in the 8th) 2, 4, 2. He then makes two pairs of throws (i. e. in all 4 throws), the first corresponding to the first parental pair and the second to the second parental pair. Suppose that he gets the holes 9, 28, 17, 12 (in the given order). The table shows that for $n_1 = n_2 = n_3 = 0$, $n_4 = 2$, $n_5 = n_6 = 0$, number 9 denotes *aa* and number 28 *AA*. The first parental pair taken from the $F_2$ is thus an $AA \times aa$ cross. Likewise 17 denotes *Aa* and 12 also *Aa*, and consequently the second parental pair in the $F_2$ is an $Aa \times Aa$ cross. The parents from the $F_2$ correspond thus to $n_1 = n_2 = 0$, $n_3 = n_4 = 1$, $n_5 = n_6 = 0$, and the table shows that in the progeny ($F_3$) the numbers of *AA*, *Aa*, and *aa* individuals are (in the 8th) 1, 6, 1. The student makes 2 new pairs of throws and, using the entry $n_1 = n_2 = 0$, $n_3 = n_4 = 1$, $n_5 = n_6 = 0$, he finds what kind of parental cross he gets in the $F_3$ and what proportions of *AA*, *Aa*, and *aa* individuals in the $F_4$. He proceeds in this way until complete fixation, which occurs when he gets either $n_1 = 2$, $n_2 = n_3 = n_4 = n_5 = n_6 = 0$ (fixation with *AA* individuals) or $n_1 = n_2 = n_3 = n_4 = n_5 = 0$, $n_6 = 2$ (fixation with *aa* individuals).

In the case of brother—sister matings only one pair of throws (i. e. in all 2 throws) is made per generation, and in the case of 5 parents per generation, 5 pairs of throws (i. e. in all 10 throws) are made per generation. It is to be remarked that if a certain number is not included among the holes of the roulette, as for instance zero, and if this number comes out after a throw, this throw is passed as if it had not been made.

The students have to make careful records of the results of each generation. They have also to make a number of parallel series of similar kind, just in order to study the great randomly determined variability in different series. Usually they have to make 100 series. For each generation they also make out the totals of *AA*, *Aa*, and *aa* individuals (in the 4*n*th) and divide this total by the number of series $\times 4n$, whereby they get the average proportions of the three kinds of genotypes. In the Genetics class of 1946 the students made 100 parallels of each of the cases $n = 1$, $n = 2$ and $n = 5$, and the final records of these experiments are given in Tables 3, 4 and 5. These tables show the average heterozygosity and also — and this is perhaps the most important — the total number of series which up to each generation have been fixed as *AA* or as *aa*. The result thus clearly shows: (1) The *average* heterozygosity decreases, at first at a rapid rate, later more slowly, and vanishes completely after a rather

TABLE 3. *Record for 100 series with $n = 1$.*

| Generation | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | $F_8$ | $F_9$ | $F_{10}$ | $F_{11}$ | $F_{12}$ | $F_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proportion of *Aa* | | | | | | | | | | | | |
| Observed | 0,5000 | 0,5100 | 0,3600 | 0,2900 | 0,2200 | 0,1650 | 0,1550 | 0,0950 | 0,0800 | 0,0600 | 0,0600 | 0,0450 |
| Expected | 0,5000 | 0,3750 | 0,3125 | 0,2500 | 0,2035 | 0,1641 | 0,1328 | 0,1074 | 0,0869 | 0,0705 | 0,0569 | 0,0460 |
| Total number of series which have been fixed | | | | | | | | | | | | |
| as *AA* | — | 5 | 14 | 23 | 31 | 38 | 44 | 45 | 47 | 49 | 49 | 50 |
| as *aa* | — | 7 | 15 | 21 | 24 | 30 | 31 | 34 | 36 | 39 | 41 | 41 |

| Generation | $F_{14}$ | $F_{15}$ | $F_{16}$ | $F_{17}$ | $F_{18}$ | $F_{19}$ | $F_{20}$ | $F_{21}$ | $F_{22}$ | $F_{23}$ | $F_{24}$ | $F_{25}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proportion of *Aa* | | | | | | | | | | | | |
| Observed | 0,0300 | 0,0150 | 0,0100 | 0,0100 | 0,0100 | 0,0050 | 0,0050 | 0,0050 | 0,0050 | 0,0100 | 0,0050 | 0,0050 |
| Expected | 0,0372 | 0,0301 | 0,0244 | 0,0197 | 0,0159 | 0,0129 | 0,0104 | 0,0084 | 0,0068 | 0,0055 | 0,0045 | 0,0036 |
| Total number of series which have been fixed | | | | | | | | | | | | |
| as *AA* | 52 | 53 | 53 | 53 | 53 | 54 | 54 | 54 | 54 | 54 | 54 | 54 |
| as *aa* | 42 | 44 | 45 | 45 | 45 | 45 | 45 | 45 | 45 | 45 | 45 | 45 |

| Generation | $F_{26}$ | $F_{27}$ |
|---|---|---|
| Proportion of *Aa* | | |
| Observed | 0,0050 | 0,0000 |
| Expected | 0,0029 | 0,0024 |
| Total number of series which have been fixed | | |
| as *AA* | 54 | 55 |
| as *aa* | 45 | 45 |

great but limited (as long as the number of parallel series is limited) number of generations. (2) This rate of decrease in heterozygosity is very different in different series and the variation is due to random causes. In the case of 2 parents per generation, for instance (Table 4), one series was completely homozygous already in the $F_3$, whereas one series did not get rid of its heterozygosity until the $F_{62}$. It is also due to random causes if the fixation should be in respect to *A* or to *a*. (3) *On an average* it takes more generations in the case of $n = 5$ to get completely rid of heterozygosity than in the case of $n = 2$ or

TABLE 4. *Record for 100 series with n = 2.*

| Generation | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | $F_8$ | $F_9$ | $F_{10}$ | $F_{11}$ | $F_{12}$ | $F_{13}$ | $F_{14}$ | $F_{15}$ | $F_{16}$ | $F_{17}$ | $F_{18}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proportion of *Aa* | | | | | | | | | | | | | | | | | |
| Observed ..... | 0,5000 | 0,4775 | 0,4050 | 0,3875 | 0,3300 | 0,2750 | 0,2600 | 0,2250 | 0,2125 | 0,1875 | 0,1550 | 0,1475 | 0,1450 | 0,1300 | 0,0975 | 0,0875 | 0,0750 |
| Expected ..... | 0,5000 | 0,4375 | 0,3906 | 0,3477 | 0,3096 | 0,2756 | 0,2454 | 0,2185 | 0,1946 | 0,1732 | 0,1542 | 0,1374 | — | — | — | — | — |
| Total number of series which have been fixed | | | | | | | | | | | | | | | | | |
| as *AA* | — | — | 1 | 7 | 13 | 18 | 20 | 24 | 26 | 31 | 32 | 35 | 35 | 36 | 37 | 40 | 42 |
| as *aa* | — | 1 | 5 | 9 | 13 | 15 | 21 | 23 | 25 | 25 | 30 | 30 | 34 | 36 | 39 | 40 | 40 |

| Generation | $F_{19}$ | $F_{20}$ | $F_{21}$ | $F_{22}$ | $F_{23}$ | $F_{24}$ | $F_{25}$ | $F_{26}$ | $F_{27}$ | $F_{28}$ | $F_{29}$ | $F_{30}$ | $F_{31-32}$ | $F_{33-35}$ | $F_{36}$ | $F_{37-41}$ | $F_{42}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proportion of *Aa* | | | | | | | | | | | | | | | | | |
| Observed ..... | 0,0375 | 0,0425 | 0,0375 | 0,0350 | 0,0275 | 0,0350 | 0,0250 | 0,0275 | 0,0225 | 0,0200 | 0,0150 | 0,0100 | 0,0075 | 0,0050 | 0,0075 | 0,0025 | 0,0050 |
| Total number of series which have been fixed | | | | | | | | | | | | | | | | | |
| as *AA* | 44 | 47 | 47 | 48 | 48 | 48 | 50 | 50 | 51 | 51 | 51 | 51 | 52 | 52 | 52 | 52 | 52 |
| as *aa* | 41 | 42 | 43 | 44 | 44 | 44 | 44 | 44 | 45 | 45 | 46 | 46 | 46 | 46 | 46 | 47 | 47 |

| Generation | $F_{43-53}$ | $F_{54}$ | $F_{55}$ | $F_{56}$ | $F_{57-58}$ | $F_{59}$ | $F_{60-61}$ | $F_{62}$ |
|---|---|---|---|---|---|---|---|---|
| Proportion of *Aa* | | | | | | | | |
| Observed ..... | 0,0025 | 0,0075 | 0,0100 | 0,0050 | 0,0075 | 0,0050 | 0,0025 | 0,0000 |
| Total number of series which have been fixed | | | | | | | | |
| as *AA* | 52 | 52 | 52 | 52 | 52 | 52 | 52 | 53 |
| as *aa* | 47 | 47 | 47 | 47 | 47 | 47 | 47 | 47 |

TABLE 5. *Record for 100 series with n = 5.*

| Generation | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | $F_8$ | $F_9$ | $F_{10}$ | $F_{11}$ | $F_{12}$ | $F_{13}$ | $F_{14}$ | $F_{15}$ | $F_{16}$ | $F_{17}$ | $F_{18}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proportion of *Aa* Observed ..... | 0,500 | 0,500 | 0,495 | 0,432 | 0,394 | 0,356 | 0,338 | 0,305 | 0,283 | 0,263 | 0,237 | 0,236 | 0,211 | 0,202 | 0,178 | 0,157 | 0,146 |
| Total number of series which have been fixed | | | | | | | | | | | | | | | | | |
| as *AA* | — | — | — | — | 2 | 3 | 5 | 7 | 10 | 11 | 14 | 15 | 17 | 19 | 22 | 27 | 28 |
| as *aa* | — | — | — | 1 | 2 | 4 | 5 | 9 | 15 | 16 | 19 | 23 | 25 | 26 | 29 | 31 | 32 |

| Generation | $F_{19}$ | $F_{20}$ | $F_{21}$ | $F_{22}$ | $F_{23}$ | $F_{24}$ | $F_{25}$ | $F_{26}$ | $F_{27}$ | $F_{28}$ | $F_{29}$ | $F_{30}$ | $F_{31}$ | $F_{32}$ | $F_{33}$ | $F_{34}$ | $F_{35}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proportion of *Aa* Observed ..... ..... | 0,144 | 0,134 | 0,127 | 0,115 | 0,109 | 0,116 | 0,109 | 0,088 | 0,079 | 0,075 | 0,063 | 0,066 | 0,067 | 0,058 | 0,065 | 0,043 | 0,056 |
| Total number of series which have been fixed | | | | | | | | | | | | | | | | | |
| as *AA* | 29 | 29 | 29 | 31 | 31 | 31 | 33 | 34 | 35 | 35 | 35 | 35 | 37 | 37 | 37 | 38 | 38 |
| as *aa* | 35 | 36 | 36 | 38 | 38 | 38 | 38 | 40 | 43 | 44 | 46 | 47 | 48 | 50 | 50 | 50 | 50 |

| Generation | $F_{36}$ | $F_{37}$ | $F_{38}$ | $F_{39}$ | $F_{40}$ | $F_{41}$ | $F_{42}$ | $F_{43}$ | $F_{44}$ | $F_{45}$ | $F_{46}$ | $F_{47}$ | $F_{48}$ | $F_{49}$ | $F_{50}$ | $F_{51}$ | $F_{52}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proportion of *Aa* Observed ..... | 0,058 | 0,054 | 0,045 | 0,039 | 0,040 | 0,044 | 0,049 | 0,043 | 0,038 | 0,042 | 0,035 | 0,026 | 0,027 | 0,023 | 0,020 | 0,020 | 0,015 |
| Total number of series which have been fixed | | | | | | | | | | | | | | | | | |
| as *AA* | 38 | 38 | 38 | 39 | 39 | 39 | 39 | 39 | 40 | 40 | 40 | 40 | 40 | 42 | 43 | 44 | 44 |
| as *aa* | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 51 | 51 | 51 | 51 | 51 | 51 |

| Generation | $F_{53}$ | $F_{54}$ | $F_{55}$ | $F_{56}$ | $F_{57}$ | $F_{58}$ | $F_{59}$ | $F_{60}$ | $F_{61-64}$ | $F_{65}$ | $F_{66-67}$ | $F_{68}$ | $F_{69}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proportion of *Aa* Observed ..... | 0,014 | 0,014 | 0,013 | 0,007 | 0,009 | 0,010 | 0,007 | 0,005 | 0,003 | 0,002 | 0,003 | 0,001 | 0,000 |
| Total number of series which have been fixed | | | | | | | | | | | | | |
| as *AA* | 44 | 44 | 44 | 44 | 45 | 45 | 45 | 45 | 45 | 45 | 45 | 45 | 45 |
| as *aa* | 51 | 52 | 52 | 52 | 52 | 52 | 53 | 53 | 54 | 54 | 54 | 54 | 55 |

$n=1$, and also more generations in the case of $n=2$ than in the case of $n=1$. It is especially on point (2) that the teacher should lay particular stress, as it is this point which is practically impossible to demonstrate with living material or by average curves of heterozygosity. It is also these randomly caused variations which give rise to such an important phenomenon in Nature's household as the genetical drift.

Concerning point (1), it usually seems quite enough to emphasize the general trend in heterozygosity, whereas any comparisons between observed and expected frequencies seem, from a teacher's point of view, to be of less importance. Such expected frequencies are, however, given in Tables 3 and 4. In the case of $n=1$ (brother—sister matings) they may be found, for instance, in WRIGHT's work (1921 b, p. 127) or in JENNINGS (1914). As will be seen there, the proportion of heterozygosity in the $F_h$ is $\frac{x_h}{2^h}$, where $x_h =$ FIBONACCI's number defined by the relations $x_h = x_{h-1} + x_{h-2}$ and $x_0 = x_1 = 1$. A general formula from which it would be theoretically possible to compute these frequencies for any value of $n$ is easy to find, but it is of very limited use, as the computational difficulties increase enormously already when $n>2$. But for the sake of completeness the deduction of the formula will be shown here.

Suppose the frequencies of the zygotes $AA$, $Aa$ and $aa$ to be $x$, $y$ and $z$ $(x+y+z=1)$. Then the 6 possible modes of pairings are obviously expected in the frequencies $(x+y+z)^2 = x^2$, $2xy$, $2xz$, $y^2$, $2yz$, $z^2$. Consequently, $n$ pairs must be distributed as the expansion of $(x^2+2xy+2xz+y^2+2yz+z^2)^n$, i. e. the frequency of the combination $n_1$, $n_2$, $n_3$, $n_4$, $n_5$, $n_6$ of the 6 possible pairings must be

$$\frac{n!}{n_1!\,n_2!\,n_3!\,n_4!\,n_5!\,n_6!}\,(x^2)^{n_1}(2xy)^{n_2}(2xz)^{n_3}(y^2)^{n_4}(2yz)^{n_5}(z^2)^{n_6} =$$

$$= \frac{n!\,2^{n_2+n_3+n_5}}{n_1!\,n_2!\,n_3!\,n_4!\,n_5!\,n_6!}\; x^{2n_1+n_2+n_3}\,y^{n_2+2n_4+n_5}\,z^{n_3+n_5+2n_6}$$

If thus $p^{(h)}_{n_1 \cdots n_6}$ denotes this frequency in the generation $h$, the following formula of recurrence must hold good

$$p^{(h)}_{n_1 \cdots n_6} = \frac{n!\;2^{n_2+n_3+n_5}}{n_1!\,n_2!\,n_3!\,n_4!\,n_5!\,n_6!} \times$$

$$\times \mathrm{S}\, p^{(h-1)}_{s_1 \cdots s_6}\, x^{2n_1+n_2+n_3}\, y^{n_2+2n_4+n_5}\, z^{n_3+n_5+2n_6} \quad \ldots . \quad (2)$$

Here $x$, $y$, $z$ have the values

$$x = \frac{4s_1 + 2s_2 + s_4}{4n}$$

$$y = \frac{2s_2 + 4s_3 + 2s_4 + 2s_5}{4n}$$

$$z = \frac{s_4 + 2s_5 + 4s_6}{4n}$$

and the summation is to be made for all the $\frac{(n+5)!}{n!\ 5!}$ combinations of $s$'s for which $s_1 + s_2 + s_3 + s_4 + s_5 + s_6 = n$. The chief difficulty in the computations comes from the largeness of $\frac{(n+5)!}{n!\ 5!}$.

From the frequencies (2) the expected heterozygosity may be directly computed, and this has been done in Table 4 up to the $F_{13}$.

It is easy to construct working tables of similar kinds in order to demonstrate other effects of breeding in small populations. Thus, the Genetics class usually also makes roulette trials to demonstrate the combined effect of inbreeding and complete selection against a recessive.

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## LITERATURE CITED.

1. JENNINGS, H. S. 1914. Formulae for the results of inbreeding. — Amer. Nat. 48: 693—696.
2. WRIGHT, SEWALL. 1921 a. Systems of mating. II. The effects of inbreeding on the genetic composition of a population. — Genetics VI: 124—143.
3. — 1921 b. Systems of mating. V. General considerations. — Genetics VI: 167—178.
4. — 1931. Evolution in Mendelian populations. — Genetics XVI: 97—159.

# THE INHERITANCE OF THE PLATINUM AND WHITE FACE CHARACTERS IN THE FOX

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## INTRODUCTION.

THE black fox, which is farm bred in the Scandinavian countries, is descended from animals imported from North America, where it has arisen from the wild red fox through mutation. The mutant gene in the homozygous condition changes the colour of the whole animal, in the same way as a mutation of the agouti gene in the wild type rabbit changes the colour of the whitish underline and the banded guard hairs to solid black. Two phenotypically similar but genetically different mutant types of black foxes are known, viz. the Alaskan and the standard fox. When Alaskan blacks are crossed with reds the $F_1$ animals are intermediate in colour, showing the typical »cross fox» character, but the standard black $\times$ red foxes produces a smoky red fox, where the colour of the red fox is strongly, but not completely, dominant. Crosses between Alaskan and standard foxes produce »blended cross foxes» with characters from both of the first mentioned hybrids. The genetics of these crosses is explained by WARWICK and HANSON (ASHBROOK, 1937) as follows: *AABB* red fox, *aaBB* Alaskan black fox, *AAbb* standard fox, *aABB* cross fox, *AABb* smoky red hybrids, *AaBb* blended cross foxes, etc. Breeding data seem to fit in very well with this hypothesis.

The modern *silver fox* is a product of selection from the original, almost wholly black, mutants. The silvering is caused by an intermingling of light banded guard hairs among the black hairs. The wild red fox has such silver hairs in varying numbers, particularly on the rump and loin, and some silver hairs are found even on the most genuine black foxes. The silvering is a quantitative character which is present in both red and black foxes, but the black fox and its mutants, e.g. white face and platinum, which will be discussed later, are qualitatively different from one another and from the red fox.

The common red fox, which ranges over the northern parts of

America, Europe and Asia, ought to be considered as belonging to one and the same species, *Vulpes vulpes* L., as pointed out by COLE and SHACKELFORD (1943). Local varieties or breeds can easily be distinguished, but, owing to the fact that all these varieties freely interbreed as soon as opportunity offers, there seems to be no logical reason to speak of different species. Mutant black foxes have also appeared from time to time among the wild living Scandinavian red foxes, and some of these mutants have been kept in captivity long before any importations of North American silver foxes were made. Such mutants have not been utilized, however, to build up any strains of our present day farm foxes. Sometimes the breeders may use Scandinavian red foxes for crossing with the farm-raised silver foxes, and in this way some of the local »*vulpes* blood» may find its way into the breeding stock. The Scandinavian silver fox breeding is, however, almost exclusively founded on imported animals, viz. the Canadian standard silver fox, probably due to the fact that skilled fox farming with selection for high quality pelts and extensive silvering started much earlier in North America than in the Scandinavian countries.

In Canada and Northern U. S. wild living black foxes and their cross products with the red fox seem to be of rather frequent occurrence, but in Sweden they are exceptional. BUTLER (1945) has studied the data on more than 700.000 Canadian fox pelts of the Hudson's Bay Company fur returns 1915—1944. He found the highest percentage of silver fox pelts in British Columbia (8,0 % silver and 41,3 % hybrids) and the lowest in Saskatchewan (3,0 % silver and 24,6 % hybrids). The frequency of silver foxes has decreased during the last 30 years from about 9 to 3 per cent. Three possible explanations of this decrease are put forward, viz. migration, differential survival or reproductive rates, and changed classification standards. WARWICK and HANSON's factorial hypothesis fitted well to the observed frequencies of the different colour phases.

## NEW MUTANT COLOUR PHASES OF THE SILVER FOX.

### A REVIEW OF THE LITERATURE.

Several new colour phases have appeared among farm bred silver foxes, and three such mutants are well described in the literature, viz. the white face, platinum and pearl platinum fox. The last-mentioned will be referred to here simply as the pearl fox, because it has nothing to do with the platinum character, and the term pearl platinum is apt to cause

confusion (BOWNESS, 1944). The platinum fox is in the U. S. usually called »platinum silver» or »Norwegian platinum», in Canada »standard platinum», and in the Scandinavian countries »Mons-platinum» — after the original mutant — or simply platinum. The latter term would seem to be adequate. Different names are also used for the white face fox, e.g. »white-marked silver», »ring-neck» or »platinum type silver», while in Norway it is spoken of as »Hovbrender platinum». Any name of the white face fox, which involves the term »platinum» should, however, be avoided.

The platinum fox is well described by MOHR and TUFF (1939) and by COLE and SHACKELFORD (1943). The latter authors have also fully described the white face fox. A brief characterisation here of these mutants will, therefore, be sufficient.

The *white face fox* is very similar to the common silver fox, except that it has more or less extensive white markings on the nose, forehead, neck, feet, breast and belly; the tip of the tail is white to the same extent as in the silver fox. The pigmentation of the underfur, the guard hairs and the ears, trunk and tail has about the same intensity as in the silver fox. There is no sharp borderline between the silver and the white face fox; the former may have white markings on the feet, breast and belly, and the latter may lack the white blaze along the nose, and the white collar (Fig. 1). There is the possibility, therefore, that the classification of the two types may sometimes be wrong. The variation in markings and shade of colour is great, and there may be some overlapping at the other end of the scale, towards the platinum fox. The light white face fox often shows a kind of roaning, owing to the presence of numerous wholly white guard hairs among the banded silver hairs. These white hairs give to the fur a »dead» appearance, and the lustre of the fur will not be the same as in the true silver fox. COLE and SHACKELFORD describe the white face foxes as »silvers on which the characteristic white-face markings have been superimposed».

The white face mutation seems to have occurred independently a number of times in U. S. A., Canada and Scandinavia. COLE and SHACKELFORD speak of the Colpitts, McNeil, Garvey, Holman and Springborn strains of white face, some of which have probably descended from separate mutations. The Colpitts strain seems to be the oldest one; it is said to have originated in 1928. The »Hovbrender platinum» (MOHR and TUFF, 1939) is nothing else but a white face fox which has originated in Norway. COLE and SHACKELFORD give some figures from crosses between two American white face strains, designated A and B,

which tend to show that these strains are genetically different, this being due to mutations in different loci, and perhaps different chromo-

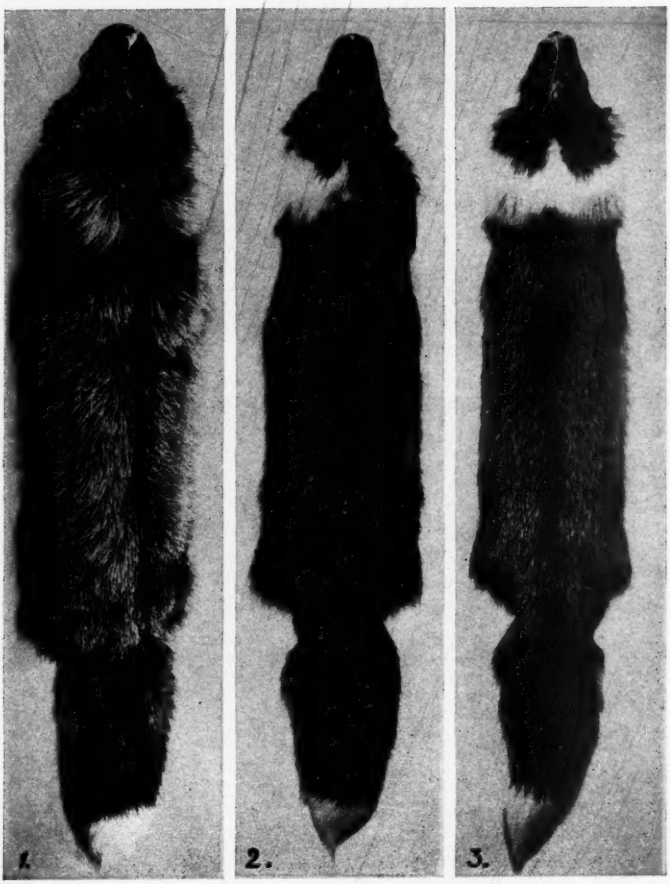


Fig. 1. Variations in the white markings of the white face fox. — To the left (1) a pelt which may be classified as silver, but it is really a white face with a small blaze and white markings on the feet and underline. — In the middle (2) a very heavily pigmented white face pelt with narrow blaze and broken collar. — To the right (3) a typical white face pelt with a narrow blaze, fairly broad and symmetrical collar, and the same intensity of pigmentation along the back and tail as in the silver fox.

somes. When the two strains are crossed with one another, the offspring shows more extensive white markings than either of the parent

strains, the white extending up on the middle of the ear while this is never the case in the common white face.

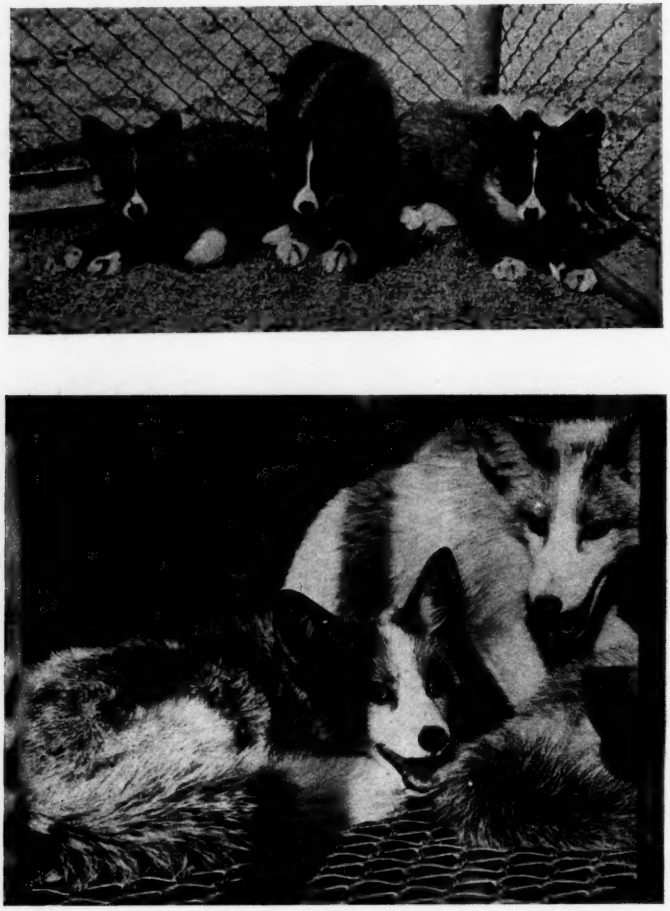

Fig. 2. Fox whelps of typical white face (above) and platinum (below) character.

The white face foxes on Swedish fox farms derive from Canada, perhaps mostly from the McNeil strain, and from Norway. The writer does not know of any original mutations in Sweden, and all our white face foxes would seem to be identical as far as the genotype for this particular character is concerned.

The *platinum fox* has a dilute pigmentation. Its underfur is distinctly lighter than that of the silver and white face foxes, the colour ranging from white to greyish; many guard hairs are entirely unpigmented, and others are pigmented only below the tapered end. The white blaze, the white collar around the neck, and the white markings on the feet, breast and belly are usually much more extensive than in white face foxes, and the ears are much lighter coloured; the blaze is as a rule narrow in a white face fox but broad in a platinum (Fig. 2). There cannot be any mistakes in the classification of platinum and silver foxes, but the variation of platinum and white face may, to some extent, overlap. By the lighter ears and underfur and the broader blaze it should be possible, however, to distinguish the true platinum fox. COLE and SHACKELFORD (1943) point out that it is possible that some of this variability is non-genetic, but they state that »it is more probable that much of it is determined by factors in the silver foxes to which the platinums are bred». The usual variation in the pigmentation of Swedish platinum foxes is shown in Fig. 3.

The first known platinum mutant appeared in Troms in Northern Norway, where the famous platinum male »Mons» was born in 1933 (MOHR and TUFF, 1939). According to COLE and SHACKELFORD (1943), at least three independent platinum mutations have occurred in the U. S. and Canada, originating the Corbin, the Cody and the La Forrest strains. All these four platinum strains are phenotypically very similar, and there is no evidence so far to show that they are genetically different. The platinum foxes which are found in Swedish fox farms originated, as far as the writer is aware, from Norway. We have no records to show that independent platinum mutations have arisen in Sweden, whereas in Norway several platinum mutations seem to have occurred.

Original data on *the inheritance of the white face and platinum characters* have been published by MOHR and TUFF (1939), COLE and SHACKELFORD (1943) and GUNN (1945). MOHR and TUFF concluded that the platinum character is inherited as an autosomal dominant. They suggested that the mutant gene is lethal in a homozygous condition, but the data in support of this suggestion were admittedly very meagre. COLE and SHACKELFORD reached the conclusion, however, that the suggestion as to the lethality of the platinum gene was correct, and that the mutant white-face gene behaves in exactly the same manner as that of the platinum. They suggested that the genes for silver, white face and platinum are allelic to one another, any of the possible

three combinations of these mutant genes being lethal. Completely white whelps with blue eyes were occasionally found in the newly born litters after matings of mutant × mutant, and the authors assume that these whelps represent the homozygous dominant type, which is

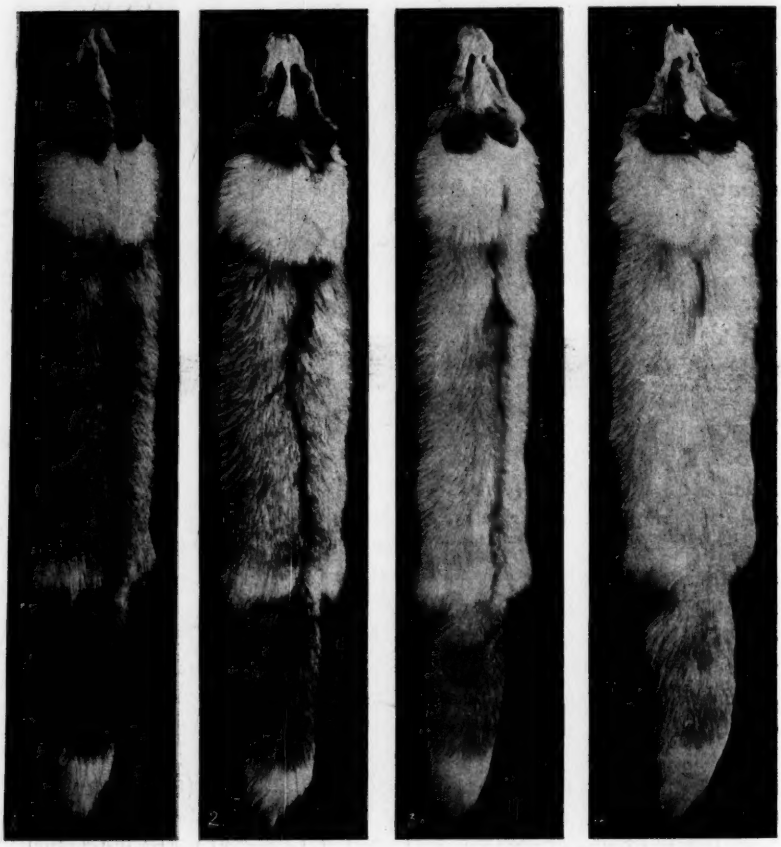

Fig. 3. Variations in the intensity of pigmentation of Swedish platinum foxes. Darker platinum pelts than the darkest one above (1) may be found.

not viable. One such white whelp lived, however, to about five weeks of age. Three white whelps after matings of platinum × platinum, listed in Table 1, were all borne by the same vixen and sired by the same male. The litter size in COLE and SHACKELFORD's matings of white face × platinum is very high and does not lend support to the hypo-

thesis that the genes for these two characters are allelic with lethal effect when combined in the same zygote. Only one critical »test mating» is referred to: a platinum male, offspring from a white face × platinum cross, was mated to 3 silver females and produced 9 platinum pups, 10 silvers and no white face, i.e. the proportions expected if the male did not carry the white face gene. Furthermore, the mutational changes are depicted diagrammatically as a progressive deficiency; one

TABLE 1. *Data from crosses between mutant foxes.*

| Matings | Number of litters | Litter size | Type of offspring | | | |
|---|---|---|---|---|---|---|
| | | | Silver | Platinum | White | White face |
| *Platinum × platinum:* | | | | | | |
| MOHR and TUFF (1939) | 2 | 3,5 | 2 | 5 | — | — |
| COLE and SHACKELFORD (1943) | ? | ? | 10 | 22 | — | — |
| | 11 | 2,1 | 8 | 12 | 3 | — |
| Total | | | 20 | 39 | 3 | |
| *White face × white face:* | | | | | | |
| MOHR and TUFF (1939) | 3 [1] | 4,0 | 4 | — | — | 8 |
| COLE and SHACKELFORD (1943) | 2 [2] | 4,5 | 1 | — | 5 | 3 |
| | 79 | 3,3 | 79 | — | — | 183 |
| GUNN (1945) | (141) [3] | 3,5 | 115 | — | — | 238 |
| Total | | | 299 | — | 5 | 432 |
| *White face × platinum:* | | | | | | |
| COLE and SHACKELFORD (1943) | 6 | 6,1 | 15 | 16 | — | 6 |
| GUNN (1945) | (14) [3] | 3,1 | 12 | 16 | — | 16 |
| Total | | | 27 | 32 | — | 22 |

[1] »Hovbrender platinum». [2] Selected litters where white pups were produced. [3] Number of matings.

break at the end of the chromosome causes the white face character, and a second break the platinum. A viable fox must have at least one of the homologous chromosomes intact. GUNN presents further evidence as to the non-viability of the homozygous mutants, but he does not assume that the genes for white face and platinum are allelic.

The data from crosses between the mutant types, on which the previous workers have based their conclusions, are summarized in Table 1.

The combined data from the three sources are still rather meagre, and the results from the white face × white face and the white face ×

platinum matings do not agree too well with the 1 : 2 and the 1 : 1 : 1 ratios, which are expected on the basis of the proposed hypothesis.

The third mutant is *the pearl fox*, which is recessive to silver, or black. The first animals of this type were rapported from a farm in Minnesota, U. S. A., in 1934 (BOWNESS, 1944). The pearl fox has an even blue-greyish colour throughout and no white markings, except the white on the tip of the tail and the white bands on the silver hairs. Two genetically different strains are known, the Ontario and the New Brunswick strains (DEAKIN, 1942; GUNN, 1945), which when crossed with one another produce only silver foxes, heterozygous for both the pearl genes. Breeding within the same strain yields only pearl foxes. When pearl foxes are mated to white face foxes, 50 % white face and 50 % silver pups are obtained. These white face foxes carry the recessive gene for the pearl character, and when they are mated to homozygous pearl foxes 25 % silver, 25 % white face, 25 % pearl and 25 % *pearlatina* pups are obtained. The pearlatina is a pearl fox with white face markings. *Glacier blues* are produced in the same way by using platinum for the cross instead of white face (COLE, 1945). Glacier blues are dilute pearl foxes with platinum markings. As far as the writer knows, the pearl fox does not exist in the Scandinavian countries.

## OWN INVESTIGATIONS.

The present writer has made an effort to analyse the inheritance of the white face and platinum mutants. Two different lines of approach have been followed; statistical data, compiled from Swedish fox farms, were analysed, and in the breeding season of 1946 an experimental inquiry was made regarding the intra-uterine development of the pups of platinum parents. The statistical work may be considered as completed, but the experimental breeding will be continued next season, and probably will have to be continued for several years before the problem of what happens to the homozygous mutants is solved.

### AN ANALYSIS OF BREEDING DATA FROM SWEDISH FOX FARMS.

At the end of each breeding season the members of the Swedish Fur Breeders' Association report all matings on their respective fox farms to the office of the association. About the first of August all whelps in the litters are ear marked by tattooing, and a report on the number of young born in each litter, and also the ear number, the colour phase, and the parents of each whelp which is alive at the date

TABLE 2. *Reproductive results of different mating combinations.*

| Matings | Total number of mated vixens | Empty vixens | | Abortions | | Number of litters | | | Number of pups born in litters where a count was made | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Number of pups in the litter | | | | | Mortality during the first three months | |
| | | | | | | Not counted [2] | | Counted | | | | |
| | | Number of vixens | Per cent of total vixens [1] | Number of abortions | Per cent of pregnant vixens [1] | Number of litters | Per cent of total litters [1] | Number of litters | Total pups | Average litter size | Total pups dead | Per cent |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Silver × silver | 6.280 | 223 | 3,55 | 56 | 0,92 | 156 | 2,60 | 5.845 | 26.165 | 4,48 | 2.536 | 9,69 |
| Silver ♀ × white face ♂ | 1.029 | 41 | 3,98 | 7 | 0,71 | 32 | 3,26 | 949 | 4.228 | 4,46 | 365 | 8,63 |
| Silver ♀ × platinum ♂ | 1.911 | 74 | 3,87 | 13 | 0,71 | 58 | 3,18 | 1.766 | 7.975 | 4,52 | 1.164 | 14,60 |
| Silver ♂ × white face ♀ | 671 | 58 | 8,64 | 28 | 4,57 | 49 | 8,38 | 536 | 2.358 | 4,40 | 330 | 13,99 |
| Silver ♂ × platinum ♀ | 201 | 20 | 9,95 | 10 | 5,52 | 14 | 8,19 | 157 | 667 | 4,25 | 154 | 23,09 |
| White face × white face | 286 | 25 | 8,74 | 11 | 4,21 | 26 | 10,40 | 224 | 808 | 3,61 | 158 | 19,55 |
| White face ♀ × platinum ♂ | 159 | 20 | 12,58 | 1 | 0,72 | 9 | 6,52 | 129 | 473 | 3,67 | 61 | 12,90 |
| White face ♂ × platinum ♀ | 27 | 3 | 11,11 | 1 | 4,17 | 1 | 4,35 | 22 | 71 | 3,23 | (2) | (2,82) |
| Platinum × platinum | 75 | 10 | 13,33 | 2 | 3,08 | 5 | 7,94 | 58 | 190 | 3,28 | 28 | 14,74 |

[1] The percentages are somewhat too low throughout owing to the omission of most of the vixens, which were culled because of breeding failures at one year of age. In comparing the different classes with one another the percentages may be supposed, however, to give a reliable indication of existing inter-class differences.

[2] All pups in the litters died early after birth.

of tattooing is sent to the office. The second report can be checked against the first one as to the parentage of the litters. The pups in the litters are usually counted within a few days after birth, but on some farms the count may be made as late as a week after parturition. Notes are not always made at the same time, however, on the type of pups in the litters. Therefore the data are incomplete as to the type of the pups which have died before they are tattooed. On the following pages the deaths between birth and tattooing are referred to as »mortality during the first three months after birth». This time interval of three months is, however, only an approximate average from which the age of an individual litter may deviate rather considerably.

The breeding data from the sources stated were transferred to mimeographed cards. One card was written out for each vixen, so that a card would give the whole reproductive history of the vixen. Most of the cards, and in every case those with data on matings of mutant × mutant, were afterwards checked on the fox farms in order to avoid, as far as possible, omissions and errors. The result of the analysis of this material is presented in Tables 2, 3 and 4.

The most pertinent figures of Table 2 in relation to our present problem are those in col. 10 giving the average litter size from the different mating combinations. The summarized results are as follows:

| Matings | Total litters | Average litter size | Standard deviation of the litter size |
|---|---|---|---|
| Silver × silver ..... | 5.845 | $4{,}48 \pm 0{,}0182$ | 1,394 |
| Silver × mutant ..... | 3.408 | $4{,}47 \pm 0{,}0242$ | 1,411 |
| Mutant × mutant..... | 433 | $3{,}56 \pm 0{,}0636$ | 1,323 |

The matings of silver × silver and silver × mutant have produced litters of practically identical average size, but after matings of mutant × mutant the litters are reduced by 20,4 per cent of what may be considered their normal size. The difference in litter size between mutant × mutant and the other two types of matings is highly significant. In Table 2 it is seen that the litter size from matings of silver ♂ × mutant ♀ is a little lower than from the reciprocal mating, mutant ♂ × silver ♀, and it seems likely that the difference can be explained by the higher mortality in litters from vixens of the mutant types. In matings of mutant × mutant the average litter size from white face vixens is $3{,}63 \pm 0{,}0713$ but from platinum vixens only $3{,}26 \pm 0{,}1353$. The former average is higher, but the latter is a little

lower than would be expected if 25 per cent of the pups in litters from mutant × mutant matings die in the uterus, or immediately after birth, and thus escape counting. The expectation under such circumstances would be $4{,}48 \times 0{,}75 = 3{,}36$. The difference between the two averages is $0{,}37 \pm 0{,}1529$ or $2{,}42$ times its standard error, and the probability of obtaining this result due to chance in sampling is less than $0{,}02$.

In order to reveal, if possible, the causes of the differences in litter size of the various mating combinations, an account was made of all mated vixens and all the litters; the figures obtained are presented in Table 2. In cases where all the pups in a litter had died early after birth they were not always counted, or notes of the count were not made. This fault, as well as the differences between groups in the frequency of »empty vixens» and abortions, may affect the average litter size of the different groups. The figures presented (columns 3, 5, 7 and 12 of Table 2) do not, however, show a higher fetal or postnatal mortality after mutant × mutant matings when the mother of the litter is a platinum than when she is a white face fox. It might be assumed that the homozygous white face pups are viable in some cases, whereas the homozygous platinums are always non-viable, but this does not explain the fact that the greatest difference in litter size is found between the two reciprocal matings white face × platinum, where the pups would be supposed to be, on an average, genetically identical. Therefore, it seems most logical to assume that the actual figures for the average litter size in the four different matings of mutant × mutant are all approximations of one and the same theoretical value, i.e. 75 % of the normal litter size in matings of silver × silver or silver × mutant, the deviations being due to chance in sampling, or to differential mortality in the litters.

In Table 3 data are presented showing the type-segregation of the offspring from different matings of mutant × silver and mutant × mutant. The obtained ratios are compared to those expected according to COLE and SHACKELFORD's hypothesis that the hereditary factors for the silver, white face and platinum characters form a series of multiple alleles, here denoted $W^+$, $W$ and $W^P$, and that all the three possible combinations of two of the mutant genes in one individual, i.e. $WW$, $WW^P$ and $W^PW^P$, are lethal. The significance of the deviation of obtained from expected frequencies is tested by PEARSON's $\chi^2$ test, and the P value corresponding to each computed $\chi^2$ is given in the table (FISHER, 1932). There is a good agreement between the obtained and the expected frequencies except in the silver × platinum matings,

TABLE 3. *Mendelian segregation in the offspring from different mating combinations, and early mortality of silver, white face and platinum pups.*

| Matings | Total number of pups of known type | | | | | | Mortality during the first three months after birth | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Actual numbers | | | Ratios | | | Number of pups dead before three months of age | | | | | Per cent of total pups of known type | | |
| | | | | | | | Type unknown | | Type known | | | | | |
| | Silver = S | White face = W | Platinum = P | Obtained | Expected | P [1] | Number of pups | Per cent of total pups born | Silver | White face | Platinum | Silver | White face | Platinum |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Silver × platinum..... | 4157 | — | 3842 | 1S : 0,92P | 1S : 1P | 0,0001<br>0,15 [2] | 556 | 6,5 | 258 | — | 504 | 6,2 | — | 13,1 |
| Silver × white face..... | 3038 | 2986 | — | 1S : 0,98W | 1S : 1W | 0,50 | 256 | 4,1 | 201 | 238 | — | 6,6 | 8,0 | — |
| White face × white face | 267 | 483 | — | 1S : 1,81W | 1S : 2W | 0,21 | 22 | 2,9 | 54 | 82 | — | 20,2 | 17,0 | — |
| White face × platinum . | 167 | 188 | 182 | 1S : 1,13W : 1,09P | 1S : 1W : 1P | 0,50 | 9 | 2,5 | 14 | 18 | 22 | 8,4 | 9,6 | 12,1 |
| Platinum × platinum ... | 58 | — | 127 | 1S : 2,19P | 1S : 2P | 0,35 | 5 | 2,6 | 4 | — | 19 | 6,9 | — | 15,0 |
| Total and averages | 7687 | 3657 | 4151 | — | — | — | 848 | 5,2 | 531 | 338 | 545 | 6,9 | 9,2 | 13,1 |

[1] Probability that the deviation between the obtained and the expected ratios may be due to chance in sampling (PEARSON's $\chi^2$ test).

[2] P is calculated on the assumption that, of 556 pups of unknown type, 66,14 % were platinums, as is the case among the dead pups of known type.

where a highly significant discrepancy is noted. We think, however, that this discrepancy can be explained on the basis of the following two facts: (1) the frequency of dead pups of unknown type is very much higher in this class of matings than in any of the other four classes, and (2) the mortality of the platinum pups is almost twice as high as that of the silvers. The rather pronounced difference between the classes in percentage pups of unknown type is probably due to the data not having been random samples. The mating silver ♀ × platinum ♂ is used most extensively, by skilled and unskilled fox farmers, but the mating of mutant × mutant has been practised almost exclusively by skilled breeders, who keep more accurate and complete records than their less skilled colleagues. The mating silver ♀ × white face ♂ is also widely practised, but here the difference in death-rate between the two classes of pups is comparatively small.

If we assume the proportion of silvers to platinums among the 556 pups of unknown type which perished early from the silver × platinum matings to be the same as that of the pups of known type from the same mating, i.e. 258 silvers : 504 platinums, or 66,14 % platinums, then we would classify 188 pups of the unknown type as silvers and 358 as platinums. The obtained ratio would then be (4157 + 188) : (3842 + 368), or 1 silver : 0,97 platinum, and the P value would rise to 0,15. Under this supposition the obtained ratios in all mating combinations are in good agreement with the expected ratios.

It seems likely that the agreement between the obtained and the expected frequencies would have been still better if the pups had been counted and classified in all litters immediately after birth. Table 2 presents the frequency of litters where no count, or classification, of the pups was made. Because of the excessive mortality of platinum pups it may be expected that their proportion in these lost litters was relatively high. Furthermore, as the count and classification are often postponed to several days after the birth of the litter, it may be expected that a number of pups die and are eaten by the mothers before any notes of their existence are made. In order to obtain the exact frequencies of the pups in all classes, the vixens must be carefully watched at the time of parturition, and immediate counts and classifications must be made.

The writer has tried to test the previously mentioned hypothesis also by a search for such foxes from mutant × mutant matings as have been used for breeding and have produced offspring. The breeding history of such foxes was investigated, and the following results obtained.

(1) Eleven platinum foxes from platinum parents were found. Six of these foxes were mated to silvers and produced in 20 litters, 41 silver and 32 platinum pups. All six foxes produced silver pups and were accordingly heterozygous for the platinum character. The other 5 platinum foxes were mated to platinum and white face. Three of these foxes threw silver pups and a fourth a white face, and consequently they were also heterozygous, but one after mating to platinum produced only 4 platinum pups, and this fox might have been homozygous. On a chance basis we would expect, however, to obtain such a result in one trial out of five when heterozygous platinums are mated to one another.

(2) Eight white face foxes from white face parents were mated to silvers and produced in 8 litters, 18 silver and 17 white face pups. One of the eight litters contained only silver and one only white face pups. Three white face foxes from white face parents were mated to white face and produced in each one of 3 litters both silver and white face pups. Of these 11 white face foxes from white face parents 10 were certainly, and one probably, heterozygous for the white face character.

(3) Three platinum and two white face foxes from platinum × white face matings were found. Their breeding results are presented in Table 4.

TABLE 4. *Breeding tests of offspring from matings of platinum × white face.*

| Tested animals | Mated to | Total litters | Number and type of pups | | | |
|---|---|---|---|---|---|---|
| | | | Total | Silver | White face | Platinum |
| Platinum D 202 ♂... | 9 silver ♀♀ | 9 | 39 | 26 | — | 13 |
| » B 143 ♀ ... | 3 silver ♂♂ | 3 | 17 | 8 | — | 9 |
| » H 62 ♂ ... | 2 silver ♀♀ | 2 | 8 | 6 | — | 2 |
| White face D 15 ♀... | 1 silver ♂ | 1 | 3 | 2 | 1 | — |
| » D 15 ♀... | 1 white face ♂ | 1 | 3 | 1 | 2 | — |
| » B 27 ♀... | 2 white face ♂♂ | 2 | 9 | 3 | 6 | — |

Homozygous platinum foxes have never been found by previous workers, and they are not found in our material. As to the white face, KELLOGG (1946) states: »In other herds it has been reported that a certain strain of white marked animals when mated repeatedly to silver foxes produce litters with 100 per cent white marked progeny. This

would seem to be sufficient evidence that the homozygous white marked fox can be produced». In the U. S., where COLE and SHACKELFORD's (1943) data indicate that two genetically different strains of white foxes may occur, it would be possible that the homozygous mutants are non-viable in only one of these two strains. In Sweden we have no records of homozygous white face foxes, and our own data point rather decisively towards a general lethality of this gene combination. All available facts fit in with the hypothesis on the allelic nature of the white face and platinum genes. A crucial test of this hypothesis is to breed platinum offspring from platinum × white face matings with silver foxes. If the genes for the platinum and white face characters are allelic, and their combination in the same zygote is lethal, any platinum fox of the stated parentage should, mated to silvers, produce only silver and platinum, but never white face offspring. Furthermore, any white face fox with the parentage white face × platinum should, when mated to silver or white face, produce only these two types of offspring and never platinum. In Table 4 three platinum and two white face foxes obtained from platinum × white face matings are thus tested. The number of tested animals is, of course, too small to allow definite conclusions, but as far as the breeding results go, they point towards the allelic gene hypothesis [1].

Our data show quite conclusively that the heterozygous white face and platinum foxes are less viable than the silver fox, and that the reduction in viability is progressive in exactly the same way as the mutational change in the pigmentation of the animals: $W^+ \rightarrow W \rightarrow W^P$. The pup mortality during the first three months after birth is for silver 6,91 %, white face 9,24 % and platinum 13,13 % (Table 3), and the frequency of failures in reproduction is much higher for the mutant vixens than for silvers (Table 2). On an average the reproductive efficiency is lower for the platinum than for the white face vixens. The fox breeders know this by experience, and therefore mutant vixens are used for breeding much less frequently than mutant males. Some

[1] Since the galley proof of this paper was read the writer came across a statement by C. K. GUNN [Fur Trade Journal of Canada, 1946, 23 (6): 12] that he had on record 17 cases of matings platinum × silver foxes where typical white marked pups had appeared in the litters. On the assumption by COLE and SHACKELFORD (1943), referred to on p. 155, that two non-allelic white face mutants exist in North America, the observations reported by GUNN are not surprising, and they do not interfere with the conclusion that one of these mutants is allelic to the gene for the platinum character.

breeders have mated white face × white face and platinum × platinum in the hope of obtaining homozygous animals of the mutant types. This effort is wasted, however, and owing to the frequent breeding failures after such matings, as well as small litters and high pup mortality, the economic aspects are not promising.

## EXPERIMENTAL MATINGS PLATINUM × PLATINUM.

Through the kind co-operation of the fox breeders it was possible for the writer to obtain five one-year old platinum vixens for breeding experiments in the season of 1946. The vixens were placed on the experimental farm of the Swedish Fur Breeders' Association, where housing, feed and care were provided free of charge. The plan was to mate all the five vixens to platinum males, and to try to find out what happened to the homozygous platinum pups. On the basis of COLE and SHACKELFORD's (1943) observations, referred to on page 158, we assumed that the genetic lethality became effective rather late during the fetal development, and it was decided to kill the vixens, at least those that were mated early in the season, after 45 days of pregnancy. At this stage it should be possible to ascertain the number and type of living fetuses in the uterus, and also the number of dead and perhaps partly resorbed ones.

Unfortunately only three of the vixens mated and became pregnant. One of the empty vixens (H 199) came on heat so late in the season that no active males were available, and as to the other one (H 230), intromission was not possible in spite of vigorous attempts by several males. The vixen H 199 was sent back to the breeder, and H 230 was killed 10 days after the termination of oestrus.

Immediately after being killed the vixens were opened and their reproductive organs examined. An intersection was made in the uterine wall for each locus of fetal attachment, and the type and state of development of the fetuses were determined. Then the uterus and its content, with the placental relations between fetuses and uterus intact, were fixed in 10 % formalin for later investigations in the laboratory.

When the uteri were opened, it was found that all fetuses were living and in the same state of development. A very careful search was made later, on the formalin-fixed material, for partly resorbed fetuses, and for sites of implantation of resorbed fetuses, but nothing of this kind was found. The type of each fetus could easily be determined beyond doubt. Fig. 4 shows the three litters, photographed after the formalin fixation. The ovaries of the four vixens were sectioned, and

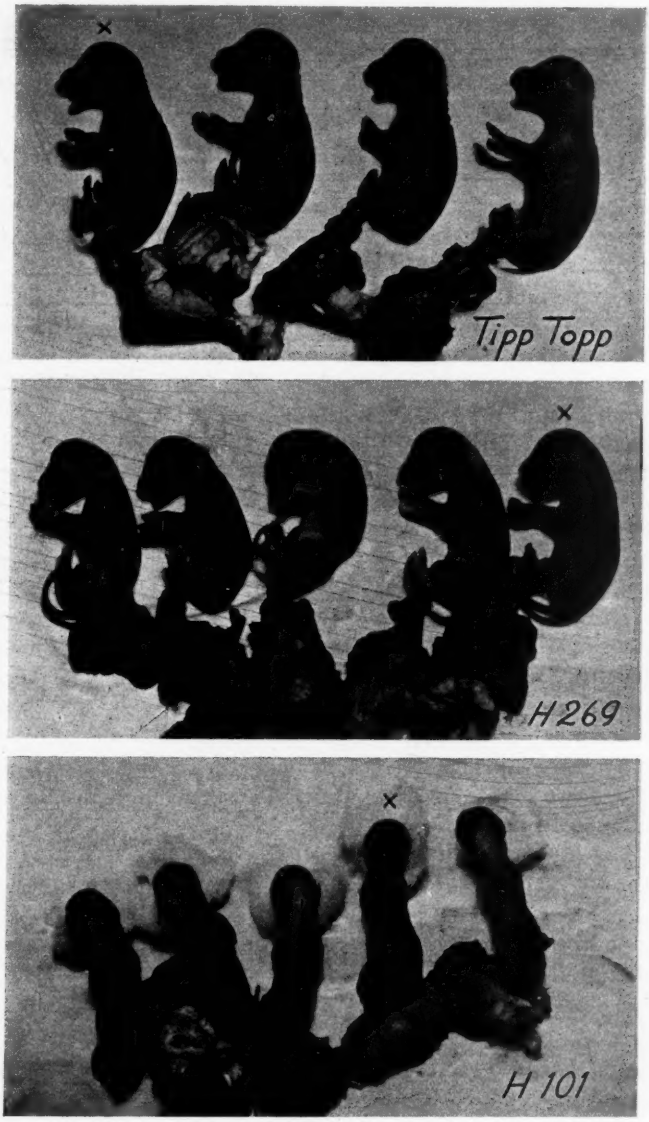


Fig. 4. Fetal litters from the breeding of platinum × platinum. The silver pups are marked with an ×.

TABLE 5. *Result of breeding platinum vixens to platinum males.*

| Experimental vixen | Date of mating | Date of killing | Pregnant days | Number of living fetuses in uterus | | | Number of corpora lutea in the ovaries |
|---|---|---|---|---|---|---|---|
| | | | | Total | Silver | Platinum | |
| Tipp-Topp | Febr. 28th | April 13th | 44 | 4 | 1 | 3 | 5 |
| H 269 | March 2nd | April 16th | 45 | 5 | 1 | 4 | 5 |
| H 101 | March 16th | April 30th | 45 | 5 | 1 | 4 | 5 |
| | | Total | — | 14 | 3 | 11 | 15 |
| H 230 | On heat March 1st and 2nd | March 11th | — | — | — | — | 5 |

the corpora lutea counted in order to ascertain the number of ova shed during oestrus. The pertinent details are given in Table 5.

The uteri of the three vixens contained 14 fetuses — 11 platinums and 3 silvers — and the total number of corpora lutea in their ovaries was 15. There were no white or only slightly pigmented fetuses, and no evidence whatever of any intra-uterine mortality. Because of (1) the normal size of all three litters and (2) the close correspondence between the number of corpora lutea and the number of fetuses, it is not probable that there had ever been any selective intra-uterine mortality. At most one zygote could have perished early because only 15 ova were shed at the time of oestrus.

The intensity of pigmentation of the platinum fetuses varied somewhat, as shown by Fig. 4, but the variation was continuous, and it was

TABLE 6. *Average weight of silver and platinum fetuses at the 45th day of intra-uterine development, and the absolute and relative weight of some of their internal organs.*

| Type of fetuses | Number of fetuses | | Average weight of fetus and organs | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Whole fetus | Lungs | Alimentary tract | Liver | Heart | Kidneys | Spleen | Thymus |
| Silver | 3 | Weight in grams | 54,68 | 2,30 | 2,47 | 4,31 | 1,23 | 0,72 | 0,12 | 0,18 |
| | | % of total weight | | 4,21 | 4,52 | 7,88 | 2,25 | 1,32 | 0,22 | 0,33 |
| Platinum | 11 | Weight in grams | 55,44 | 2,43 | 2,71 | 3,98 | 1,19 | 0,71 | 0,12 | 0,17 |
| | | % of total weight | | 4,38 | 4,89 | 7,18 | 2,15 | 1,28 | 0,22 | 0,31 |

not possible to classify any fetus as being presumably a homozygous platinum. All the 14 fetuses were weighed and dissected, and a careful search was made for anatomical abnormalities, but none was found; some of the internal organs were also weighed. We present here only the average weights of the silver and platinum fetuses. The variation within the two groups was continuous.

The differences between the averages of the two groups are not significant. No objective measures of the quantity or quality of the blood were taken, but when the formalin-fixed fetuses were cut through in the median line, after evisceration, there was a consistent and very striking difference between silver and platinum fetuses in the intensity of blood pigmentation. The platinums appeared pale and anaemic in comparison with the silvers. That anaemia is common in mutant foxes, particularly in the platinums, has been pointed out by several writers, e.g. GUNN (1946). According to TODD (1944) 10—35 % of the platinum pups are lost (U. S. A. and Canada) during the first weeks after birth, and he states that the major cause is internal hemorrhage due to lack of vitamin K. The hemorrhage is said to be more frequent among the very light platinums than among the darker ones. Also, the mature platinum foxes show, according to TODD, an increasing tendency to become anaemic with decreasing intensity of pigmentation.

The present writer will make an attempt to study the blood picture of mutant foxes in connection with future breeding experiments.

It may be taken for granted that the platinum $\times$ platinum cross is a case of monohybrid inheritance, where the mutant gene has a lethal effect in the homozygous condition. The segregation of 11 platinum : 3 silver fetuses in our breeding experiment (Table 5) may, however, be explained in three different ways. The three hypotheses are as follows.

(1) The platinum gene ($W^P$) is completely dominant over its normal allele ($W^+$) as far as its effect on the pigmentation is concerned. All fetuses are viable during the intra-uterine development, but the homozygous pups ($W^PW^P$) are non-viable after partus; as a rule they are eaten by the mother together with the placenta. According to this hypothesis the intra-uterine ratio would be 3 platinums : 1 silver, and the average number of fetuses per litter would be the same as in matings of silver $\times$ silver or silver $\times$ mutant. Post partum the ratio would be 2 platinums : 1 silver, and the litter average would be reduced to 75 % of its normal size.

(2) The lethality of the platinum gene in double dose is effective very early during the intra-uterine development and therefore no traces

of partly resorbed fetuses or sites of implantation are found. The intra- and extra-uterine ratio would be the same, i.e. 2 platinums : 1 silver.

(3) The homozygous platinum fetuses die late during pregnancy or a short time after birth. They are white in colour, and therefore it would be possible to classify them according to their genotype, as assumed by COLE and SHACKELFORD (1943). The ratio in uterus and at partus, when all fetuses, dead and living, are ascertained, would then be 1 homozygous white ($W^PW^P$) : 2 heterozygous platinums ($W^+W^P$) : 1 homozygous silver ($W^+W^+$).

The probabilities (P) of obtaining 11 platinums + 3 silvers in a sample of 14 pups on the basis of any one of the three hypothetical ratios are as follows.

| Hypothesis | Ratio | Probability *) P | P-ratios |
|---|---|---|---|
| 1 | 3 : 1 | $364\,(^3/_4)^{11}\,(^1/_4)^3 = 0{,}2402$ | $\frac{0{,}2402}{0{,}00277} = 86{,}7$ |
| 2 | 2 : 1 | $364\,(^2/_3)^{11}\,(^1/_3)^3 = 0{,}1559$ | $\frac{0{,}1559}{0{,}00277} = 56{,}3$ |
| 3 | 1 : 2 : 1 | $0{,}2402\,(^2/_3)^{11} = 0{,}00277$ | |

* The coefficient 364 is calculated by expansion of the binomial $(p + q)^{14}$.

Although our material is limited to 14 fetuses, the 1 : 2 : 1 ratio may be ruled out owing to rather poor agreement with the actual data. P of the $\chi^2$ test is less than 0,01.

Unfortunately, the number of fetuses from our experimental vixens is too small to decide between the 3 : 1 and the 2 : 1 ratio. That no evidence of intra-uterine mortality was found is, however, in favour of the 3 : 1 ratio. In the next breeding season, therefore, the writer will concentrate his efforts on a test of the hypothesis (1) that the homozygous platinums die at the time of partus, and that they are phenotypically similar to the heterozygotes as far as the pigmentation is concerned.

White or almost white pups have occasionally been found also on Swedish fox farms after matings of platinum × platinum or platinum × white face foxes, but their frequency could hardly reach more than a few per cent of the total number of pups in such litters. It seems probable that these slightly pigmented pups represent extreme segregates in a continuous series of dark to light heterozygous platinums.

The writer wishes to express his sincere thanks to the Swedish Fox Breeders' Association for the provision of working facilities at the Association's fox farm, and for all the help so readily given whenever needed in connection with the compilation of statistical data. To Mr. A. BACKHOFF, who was in charge of the work at the fox farm, thanks are due for careful observation of the experimental animals and for conscientious record keeping. Last but not least the writer is greatly indebted to those fox breeders who donated five platinum vixens for experimentation, and to all the hundreds of breeders who have willingly taken the trouble to check and complete the data on our record cards. It was this aid that made our work possible.

## SUMMARY.

Investigations were carried out on the inheritance of the white face and platinum characters, which have arisen through mutations in the silver fox. Statistical data from Swedish fox farms are presented in Tables 2, 3 and 4, showing litter size, pup mortality and Mendelian segregation in the matings of silver × mutant and mutant × mutant foxes. Experimental matings of platinum × platinum were also carried out with the object of discovering the fate of the homozygous platinum fetuses (Table 5). The results are summarized in the following paragraphs:

(1) The normal litter size in matings of silver × silver or silver × mutant was found to be 4,48, but in matings of mutant × mutant in any possible combination the litter size was reduced to 3,56.

(2) The segregation in matings of silver × mutant and mutant × mutant foxes is explained by COLE and SHACKELFORD's hypothesis that the hereditary factors for the silver, white face and platinum characters form a series of multiple alleles ($W^+$, $W$ and $W^P$), and that all the three possible combinations of the mutant genes ($WW$, $WW^P$ and $W^PW^P$) are lethal. When the differential mortality of the pups is taken into consideration our actual data fit in very well with this hypothesis.

(3) The heterozygous white face and platinum foxes are less viable than the silver fox, and the reduction in viability is progressive in the same way as the mutational change in the pigmentation. The pup mortality during the first three months after birth was for silver 6,91 %, white face 9,21 % and for platinum 13,13 %. The frequency of reproductive failures of the vixens follows the same general trend.

(4) In the experimental matings of platinum × platinum three

vixens were killed after 45 days of pregnancy. Their uteri contained 14 fetuses — 11 platinum and 3 silver — and the corresponding number of corpora lutea in the ovaries was 15. All the fetuses were living when the vixens were opened, and no evidence of any intra-uterine mortality was found. All the platinum fetuses were phenotypically alike.

(5) The results of the breeding experiment can best be explained on the basis of a 3 : 1 ratio, assuming that the homozygous mutants are viable in the uterus but die immediately after partus and are, as a rule, eaten by the mother, together with the placenta. The 2 : 1 ratio, assuming that the homozygous mutants die very early during the fetal development, cannot be rejected, however. Further experiments are needed for a decision between these two hypotheses.

Uppsala, August 22nd, 1946.

## LITERATURE CITED.


1. ASHBROOK, F. G. 1937. The breeding of fur animals. — Yearbook of Agriculture (U. S. Dept. of Agr.): 1379—1395.
2. BOWNESS, E. E. 1944. The pearl platinum fox. — Black Fox Magazine, 28 (3): 7 and 16—19, (4): 13 and 22—23.
3. BUTLER, L. 1945. Distribution and genetics of the color phases of the red fox in Canada. — Genetics, 30 (1): 39—50.
4. COLE, L. J. 1945. Breeding glacier blue and pearlatina foxes. — Nat. Fur News, 17 (1): 10.
5. COLE, L. J. and SHACKELFORD, R. M. 1943. White spotting in the fox. — Amer. Naturalist, 77: 289—321.
6. DEAKIN, A. 1942. Two different strains of pearl platinums. — Canad. Silver Fox and Fur, 8 (11): 35—38.
7. FISHER, R. A. 1932. Statistical methods for research workers. — Edinburgh, 307 pp.
8. GUNN, C. K. 1945. Genetics of some new type foxes. — Dept. of Agr., Canada. Publ. 768. 14 pp.
9. — 1946. Modification of treatment of anaemia in platinum fox pups based on trial field tests. — Amer. Fur Breeder, 18 (10): 28—29.
10. KELLOGG, CH. E. 1946. Genetics applies to fur animal breeding. — Amer. Fur Breeder: 19 (1): 6—20, and Black Fox Magazine, 1946, 29 (12): 11—13, 28—34.
11. MOHR, O. L. and TUFF, P. 1939. The Norwegian platinum fox. — Journ. of Heredity, 30: 227—234.
12. TODD, W. L. 1944. Reducing mortality in mutant foxes. — Black Fox Magazine, 27 (10): 11, 24, 25 and 27.

# SEX LINKAGE AND SEX DETERMINATION IN A MOSQUITO, CULEX MOLESTUS

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IN most animals so far studied in which sex is genetically determined there is a chromosomal difference between the sexes. One sex possesses two homologous sets of chromosomes. The other sex has one such complete set, and another set in which one or more members are absent or replaced by chromosomes of a different type. In some groups the male has only a haploid chromosome set. There are however a number of groups in which sex is wholly or mainly determined genetically, but in which no chromosomal differences between the sexes have been seen. These include all vertebrates except mammals and birds. In the *Nematocera*, the primitive suborder of the *Diptera* to which *Culex* belongs, some families have sex chromosomes, while others have not. According to the review of WOLF (1941), sex chromosomes are found in the *Phryneidae*, *Thaumaleidae*, *Scatopsidae*, and *Fungivoridae*, while no difference between the sexes has been found in *Bibionidae*, *Itoninidae*, *Culicidae*, *Melusinidae*, *Tendipedidae*, *Tipulidae*, *Limoniidae*, and *Cylindrotomidae*. In addition sex chromosomes are unknown in the *Chironomidae*. In the *Sciaridae* and *Cecidomyidae* the somatic nuclei of the sexes differ, but their germ lines do not, so that the chromosomal difference is concerned with sex development rather than determination (v. WHITE, 1945).

It has often been thought that where no sexual difference has yet been found, a further study would reveal it. However no structural differences have been discovered in the large chromosomes of the *Urodela*, or in the giant polytene chromosomes of the salivary and other glands of the *Culicidae* and *Chironomidae* (BAUER, 1935, 1936; PHILIP, 1942; SUTTON, 1942). The last author examined the giant chromosomes of the Malpighian tubules of *Culex pipiens*.

Most genetical work has been done on animals with two *X* chromosomes in one sex, and an *X* and a *Y* in the other; and there has been a tendency to regard the results obtained in them, and particularly in

*Drosophila* species, as of universal validity. In this communication we present facts which are best explained on the hypothesis that in *Culex molestus* sex is determined by a single pair of genes, maleness being dominant over femaleness, and that another gene pair located in the same chromosome shows linkage of the usual type, that is to say partial coupling or repulsion, with them.

*Systematic position and origin of stock.* — White-eyed individuals appeared in a stock of *Culex* (*Culex*) *molestus* FORSSKÅL, which is very closely allied to the commoner *Culex* (*Culex*) *pipiens* L. It was originally regarded as a biological race of *C. pipiens* in which the adults do not hibernate, the females lay fertile eggs without a blood meal, and pairing can take place in a small space. However MARSHALL and STALEY (1937) give reasons for regarding this autogenous race as a separate species, a nomenclature now generally adopted. Among other differences, *C. molestus* bites human beings far more readily than *C. pipiens*. The subject has been fully reviewed by MARSHALL (1938). It must be remembered that *C. molestus* and *C. pipiens* give fertile hybrids (TATE and VINCENT, 1936).

Our stock of *C. molestus* originated from larvae collected by Mr. P. G. SHUTE, malaria officer, Ministry of Health, from a platform sump at Old Street Underground Station, London. The stock has been maintained under laboratory conditions since March 1944 by Dr. A. BISHOP, Molteno Institute, Cambridge, whom we have to thank for supplying us with egg rafts from this stock.

In a cage containing several hundred mosquitoes 4 white-eyed females and 10 white-eyed males were found, and all other white-eyed individuals were descended from them. The eye pigment is completely lacking, and no differences in other organs or in the shape or size of the eye have been noticed. The white eye can be seen in the first larval instar and in all later stages, however the numbers in Table 1 are counts of imagines.

*Methods.* — The experimental work was entirely carried out by GILCHRIST in the Department of Entomology of the London School of Hygiene and Tropical Medicine, HALDANE being responsible only for planning and interpretation. The mosquitoes were bred in a constant temperature room at 24° C—25° C, at a relative humidity of 70 % —80 %. In these conditions the life cycle was completed in 18 to 21 days.

Larvae were reared in enamel bowls containing tap water into which food, consisting of powdered dog biscuits and stabilized wheat

embryo (»Bemax») was sprinkled. The water was changed daily and fresh food added. The first pupae appeared 10 to 12 days after the eggs hatched, and all larvae pupated within the next 3 days. Pupae were removed daily, and put into a $3 \times 1$ inch specimen tube. At this stage the normal and white-eyed individuals were separated, and the sexes were separated to a large extent. This was possible since males pupated about 24 hours earlier than females, so virgins could be obtained without isolating each individual. Up to 30 pupae could be placed in one tube without interfering with emergence. The tubes were plugged with cotton wool, and a strip of paper inserted into each which was held in place by the plug, and on which the newly hatched imagines could settle.

Paired matings were made in similar specimen tubes not more than 3 days after females had emerged. On the day after mating 1—2 cm. of water was run into the tube, and eggs were generally laid in the next 4 days. The number of eggs in a raft was counted, and it was transferred to a bowl, where the eggs hatched in 36 to 48 hours. The pre-imaginal mortality varied from about 5 % to 15 %, except in two series where it rose over 20 % owing, we have reason to believe, to accidental contamination with »D. D. T.» in dust.

*Sex ratio and single factor ratios.* — Counts of the flies derived from three rafts laid by the original white-eyed females with unknown mates suggested the form of sex linkage which was afterwards found, and systematic paired matings, with some mass matings, were undertaken. The results of all matings in which both parents were known are given in Table 1. Expectations are given in italics where segregation occurred. *w* symbolizes the recessive gene for white eye, and + its normal allelomorph; *M* symbolizes the dominant gene for maleness, and *m* its allelomorph. 36 of the 132 rafts were derived from matings of several females with one or more males. The remainder were from paired matings. The mean number of eggs per raft was 86,3. The means for normal and white-eyed mothers are 86,38 and 86,27 respectively, a very close equality.

The first two lines refer to stocks in which brother—sister mating was carried on for three generations in each case. The next two lines show the results of reciprocal crosses between them. It will be noted that the pre-imaginal mortality does not differ significantly in the three genotypes $\frac{+}{+}$, $\frac{+}{w}$, and $\frac{w}{w}$. The remainder of the flies recorded in this

TABLE 1.

| Mother | Father | Rafts | Eggs | Imagines | Normal ♀ | White ♀ | Normal ♂ | White ♂ | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| $\frac{m+}{m+}$ | $\frac{m+}{M+}$ | 9 | 702 | 636 | 310 | 0 | 326 | 0 | |
| $\frac{mw}{mw}$ | $\frac{mw}{Mw}$ | 9 | 728 | 646 | 0 | 308 | 0 | 338 | |
| $\frac{m+}{m+}$ | $\frac{mw}{Mw}$ | 2 | 137 | 124 | 61 | 0 | 63 | 0 | Normal parent from inbred line. |
| $\frac{mw}{mw}$ | $\frac{m+}{M+}$ | 2 | 138 | 118 | 53 | 0 | 65 | 0 | |
| $\frac{mw}{mw}$ | $\frac{m+}{M+}$ | 1 | 73 | 64 | 36 | 0 | 28 | 0 | Parents from $F_2$ with white grandmother. |
| $\frac{mw}{m+}$ | $\frac{mw}{Mw}$ | 4 | 297 | 262 | 69 (72) | 75 (72) | 64 (59) | 54 (59) | Mother's mother white. |
| $\frac{mw}{m+}$ | $\frac{mw}{Mw}$ | 2 | 147 | 145 | 46 (40) | 34 (40) | 33 (32,5) | 32 (32,5) | Parents from $F_2$ with white grandmother. |
| $\frac{m+}{mw}$ | $\frac{mw}{Mw}$ | 13 | 1222 | 901[1] | 233 (226) | 219 (226) | 227 (223,5) | 220 (223,5) | Mother's father withe. |
| $\frac{mw}{mw}$ | $\frac{mw}{M+}$ | 24 | 2104 | 1623 | 41 (47,1) | 711 (704,9) | 824 (816,5) | 47 (54,5) | Father's mother white. |
| $\frac{mw}{mw}$ | $\frac{mw}{M+}$ | 7 | 548 | 491 | 14 (17,0) | 257 (254,0) | 209 (206,2) | 11 (13,8) | Parents from $F_2$ with white grandmother. |
| $\frac{mw}{mw}$ | $\frac{m+}{Mw}$ | 10 | 981 | 854 | 409 (411,5) | 30 (27,5) | 39 (26,0) | 376 (389,0) | Father's father white. |
| ? $\frac{m+}{mw}$ | $\frac{m+}{Mw}$ | 1 | 86 | 79 | 36 (36,6) | 3 (2,4) | 0 (2,5) | 40 (37,5) | Parents both said to be normal with white father. |
| $\frac{mw}{m+}$ | $\frac{mw}{M+}$ | 39 | 3511 | 3331 | 851 (902,1) | 847 (795,9) | 1584 (1581,9) | 49 (51,1) | Parents' mother white. |
| $\frac{m+}{mw}$ | $\frac{m+}{Mw}$ | 9 | 722 | 631 | 267 (272,8) | 24 (18,2) | 176 (180,6) | 164 (159,4) | Parents' father white. |
| | | 132 | 11396 | 9905 | 4934 | | 4969 | | |

table were derived from earlier matings in which the normal parent was often heterozygous.

The sex ratio is very close to unity. The grand total of Table 1 is $50{,}18 \pm 0{,}50$ % of males. For the first 50 families on the record $\chi^2 = 51{,}02$, expectations being calculated on a basis of equality. This is very close to the value of 50 expected if deviations from equality

[1] Including two gynandromorphs.

were wholly due to sampling. Where the ratio is aberrant, a sex-linked lethal might at first sight be postulated. Thus one family consisted of 41 ♀, 22 ♂, and it might be thought that a lethal gene had killed off about 19 potential males. But as 63 out of 67 eggs gave imagines, this is impossible.

Before discussing the segregation of white eye we must refer to the family whose maternal origin is prefixed by a query. This family segregated as if from $\frac{mw}{mw}♀ \times \frac{mw}{M+}♂$. It is of course possible that a mistake was made as to the mother's eye colour. It is also possible that she was a mosaic, her eyes being dark, but her ovaries homozygous for *w*. All the other 109 segregating families segregated in approximately the expected ratios. Here the absence of normal males and the large number of white-eyed males are equally unexpected. This family will be referred to as the exceptional family.

The gene *w* for white eye is fully penetrant, and fully recessive on crossing. Since the sex ratio is unity we can legitimately add the data for the two sexes even when there was sex-linkage. The grand total for all back-crosses of known or presumed heterozygotes, including the exceptional family, is 2244 +, 2109 *w*, or $48{,}45 \pm 0{,}76$ % white-eyed. The ratio is much the same when the total is subdivided into groups such as the progeny of white-eyed females and normal sons of white mothers.

The offspring from all crosses of known heterozygotes *inter se* is 2878 +, 1084 *w*, or $27{,}36 \pm 0{,}71$ % white-eyed, a significant excess above 25 %. If the exceptional family is included, this becomes 27,91 %.

The single factor ratios are very steady from one family to another. Reckoning expectations of white-eyed mosquitoes as $^1/_2$ and $^1/_4$, the 19 single raft cultures from heterozygous mothers gave $\chi^2 = 11{,}23$, the 26 from heterozygous fathers gave $\chi^2 = 20{,}91$; and the 27 with both parents heterozygous gave $\chi^2 = 19{,}63$. This would be increased to 56,12 with $n = 28$, were the exceptional family included. With this exception, the variation between families is below that expected on a basis of sampling alone, though not significantly so.

*Linkage.* — Table 1 shows clearly that there is only one kind of heterozygous female, but two kinds of heterozygous male. The data agree with the hypothesis that females are *mm*, males *Mm*, and that *M* and *w* are closely linked, with a small recombination frequency *x*. Thus the two types of heterozygous male may be symbolized as $\frac{mw}{M+}$

TABLE 2.

| Mother | Father | Expected types of segregation | | | |
|---|---|---|---|---|---|
| | | $+\,♀$ | $w\,♀$ | $+\,♂$ | $w\,♂$ |
| $\frac{m+}{mw}$ | $\frac{mw}{Mw}$ | 1 | 1 | 1 | 1 |
| $\frac{mw}{mw}$ | $\frac{mw}{M+}$ | $x$ | $1-x$ | $1-x$ | $x$ |
| $\frac{mw}{mw}$ | $\frac{m+}{Mw}$ | $1-x$ | $x$ | $x$ | $1-x$ |
| $\frac{m+}{mw}$ | $\frac{mw}{M+}$ | $1+x$ | $1-x$ | $2-x$ | $x$ |
| $\frac{m+}{mw}$ | $\frac{m+}{Mw}$ | $2-x$ | $x$ | $1+x$ | $1-x$ |

and $\frac{m+}{Mw}$, according as they derived the gene $w$ from the mother or father. The expectations from the different types of segregating mating are given in Table 2.

The recombination frequency $x$ is most simply calculated from the number of cross-overs among the progeny of heterozygous males of known parentage, mated to white-eyed females. These number 113 out of 2001, giving $x = 0{,}056$. However a somewhat better estimate can be obtained from all the families showing linkage. If we assume that the mother of the exceptional family bred as $\frac{mw}{mw}$, we have $1-x : x :: 2862 : 185$; and $1-x : 1+x : 2-x : x :: 1027 : 1011 : 1851 : 73$. Applying the method of maximum likelihood, the logarithm of the likelihood is: —

$$L = 258 \log x + 1027 \log (1+x) + 3873 \log (1-x) + \\ + 1851 \log (2-x) + C.$$

Differentiating and putting $\frac{dL}{dx} = 0$, we have $7009\,x^3 - 7470\,x^2 - - 7801\,x + 516 = 0$. So $x = 0{,}0626$. If the exceptional family is assigned to its putative mother we have $7009\,x^3 - 7434\,x^2 - 7760\,x + + 516 = 0$, whence $x = 0{,}0629$. The expectations of Table 1 are calculated from the former value.

Were the uncertainty in the value of $x$ wholly due to sampling we should have for its standard error,

$$\sigma^{-2} = \frac{-d^2L}{dx^2} = \frac{258}{x^2} + \frac{1027}{(1+x)^2} + \frac{3873}{(1-x)^2} + \frac{1851}{(2-x)^2} = 71648$$

whence $\sigma = 0{,}0037$. However most of the uncertainty is not due to sampling, as appears when we calculate $\chi^2$. Taking $x = 0{,}0626$, we have $\chi^2 = 66{,}70$ for the 33 cultures in which a ratio of $1-x : x$ is expected, and $\chi^2 = 152{,}11$ for the 33 half-cultures of one sex in which a ratio of $2-x : x$ is expected. The sampling distribution of $\chi^2$ deviates from its classical form when expectations are small, but the probability that the above values should be due to sampling is extremely small. Most of the information about $x$ is derived from back-cross families, and as $\chi^2$ has twice its expected value, the amount of information is halved. The remaining information is even more reduced, so the standard error is about 0,006, or the recombination is $6{,}26 \pm 0{,}6$ %.

The cause of the divergences between different cultures is unknown. There were a few cultures with very high recombination. Thus two heterozygous brothers with white-eyed mates gave 16 cross-overs out of 99, and 12 out of 103. However brothers can differ greatly. Thus two brothers gave 11 out of 107 and 0 out of 96. The probability of obtaining so great a divergence by chance is $\frac{192!\,107!}{203!\,96!} = 6{,}94 \times 10^{-}$ Other pairs of brothers show almost as large differences. The most obvious hypothesis is that there are inversions in the neighbourhood of the genes concerned which are occasionally lost by crossing over. This is however hard to reconcile with the data, and the question can only be decided by further genetical and cytological work.

*Gynandromorphs.* — Three gynandromorphs were found, *A* in a mass culture not included in Table 1, and *B* and *C* in separate cultures from $\frac{mw}{m+}$ ♀ × $\frac{mw}{Mw}$ ♂. A search of the literature revealed two other records (MARSHALL, 1938; WEYER, 1938) of gynandromorphism in this species, and 12 in all other mosquito species both in Britain and abroad. Of these, 7 were in the genus *Aëdes* (EDWARDS, 1917; BRELJE, 1923; SHUTE, 1926; MARSHALL, 1938; SMYLY, 1942), 4 in the genus *Culex* (BEDFORD, 1914; MARSHALL, 1938; MIDDLEKAUFF, 1945), and 1 in *Theobaldia* (CLASSEY, 1942).

Our three gynandromorphs are described in Table 3. The mouth parts have not been included, as it has been found impossible to identify the stylets or their relative positions from the mounted heads. Fig. 1 shows the head of specimen *B*. Usually one appendage of a pair is

TABLE 3.

| Organ | Left side | Right side |
|---|---|---|
| *Gynandromorph A.* | | |
| Antenna | ♂ normal | Torus as in normal ♀, flagellum hairier than normal ♀, less so than normal ♂. |
| Palp | ♂ normal | Length intermediate between normal ♂ and ♀, 3rd, 4th, and 5th joints distorted, thickened, hairy. |
| Eye | Normal | Normal, slightly larger than left. |
| Fore leg, midleg | ♂ normal | ♀ normal |
| External genitalia | ♂ normal | ♀ normal |
| Gonads | Normal ovary | Normal ovary |
| Spermathecae | Two (normal ♀ has three) | |
| *Gynandromorph B.* | | |
| Antenna | ♀, hairier than normal. | ♂ normal |
| Palp | ♀ normal | ♂ normal |
| Eye | Pigmented save for 15 white facets on median posterior margin. | White, no pigment. |
| Fore leg | ♂ normal | ♂ normal |
| Midleg | 4th tarsal segment longer than 5th, claws toothed (as in ♂). | 4th tarsal segment as long as 5th, claws toothed. |
| External genitalia | ♀ normal | ♂ normal |
| Gonads | Normal testis | Normal testis. |
| Spermathecae | Two | |
| *Gynandromorph C.* | | |
| Antenna | ♂ normal | ♀ normal |
| Palp | Length between ♂ and ♀, distorted, thick, hairy. | ♂ normal |
| Eye | Pigmented | Mainly pigmented, but groups of white facets give mottled appearence. |
| Wing | Smaller | Larger |
| Fore leg, midleg | ♂ normal | ♀ normal |
| External genitalia | ♂ normal | ♀ normal |
| Gonads | One ovary, one testis, but not certain on which side situated. | |
| Spermathecae | Two | |

female, the other male, but it is not uncommon for one to be male and the other intermediate, as if a hormone diffused from the male regions. In *C* it will be noted that the right palp was male, the male appendage being on the left in other segments. The sex of the gonads has little relation to that of external organs.

Gynandromorphs *B* and *C* were members of otherwise normal cultures from $\frac{m+}{mw}\text{♀} \times \frac{mw}{Mw}\text{♂}$. Their interpretation is difficult because we do not know the sex of the eyes. Gynandromorphs in *Drosophila* generally arise by the loss of one *X* chromosome from some cells of

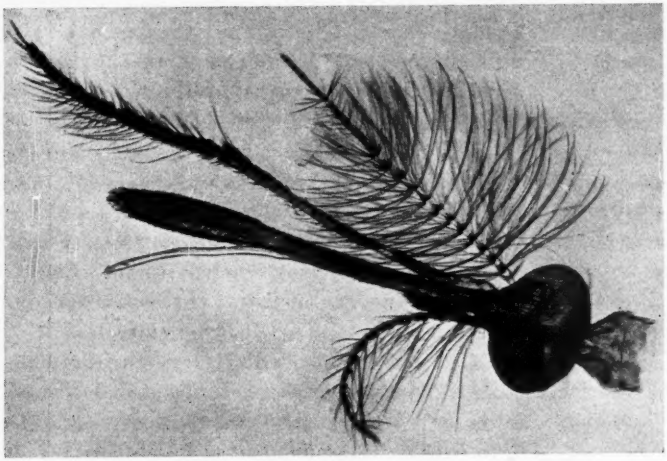

Fig. 1. Head of gynandromorph *B*. Explanation in Table 3.

what would otherwise be a female (*XX*). In a mosquito it seems quite likely that the loss of one of the six chromosomes would be fatal, and most unlikely that it would give normal female or male organs. We can at once rule out the possibility of dispermy alone. This would give a mosaic for sex but not for eye colour. On the other hand if a polar body were fertilized by a spermatozoön, and the female pronucleus by another, each providing nuclei for about half the body, the facts are explicable if one part is $\frac{m+}{mw}$ and the other $\frac{mw}{Mw}$, or if one part is $\frac{mw}{mw}$ and the other $\frac{mw}{M+}$. Such double fertilization seems to occur in Lepidoptera (GOLDSCHMIDT and KATSUKI, 1931).

If the gynandromorphs arose from a single diploid nucleus this

must have been $\frac{m+}{Mw}$. Elimination of a chromosome will not account for them unless we make the unlikely assumption that tissues with the gene *M* alone are female. But they can be explained on either of two hypotheses. Somatic crossing over may have occurred, as it does in *Drosophila*, giving rise to $\frac{m+}{m+}$ and $\frac{Mw}{Mw}$ regions. Or the two daughters of one chromosome may have gone to one pole at an early mitosis, those of its homologue going to the other. It is clear that such an event would be facilitated by somatic pairing. Either hypothesis implies that $\frac{M}{M}$ is viable, as it is in *Ambystoma*, and that the white eye regions were male. This would have been in agreement with the appendages in *B* and with the palp in *C*. When genes appear which affect sexually dimorphic organs it will be possible to decide between these hypotheses.

*The influence of eye colour on behaviour.* — Normal imagines show a well-marked reaction when an opaque object such as a hand or a piece of paper is passed between the source of light and the cage. Mosquitoes which have settled on the netting of the cage at once become very active, making a loud buzzing when in flight. The reaction is of short duration but striking in intensity. In contrast, white-eyed imagines show no such activity.

When a bright light was switched off leaving a dimly lit room the normal imagines showed no such activity. The reaction is thus one to the moving contour between a bright and dark area of the visual field, and not to a decrease in light intensity.

However preliminary experiments by Mr. T. R. RAO in the Department of Entomology of the London School of Hygiene and Tropical Medicine have shown a response of both normal and white-eyed *C. molestus* to light intensity. Two mosquito cages, one covered with black paper, were placed about 12 inches apart in a room in daylight, and connected by a cardboard tunnel. About 100 mosquitoes, male and female, were put into the darkened cage, and the number of mosquitoes which had entered the lighted cage was counted at intervals. During the daytime both types showed little activity, and remained in the darkened cage. At dusk most of them came out into the uncovered cage. The difference in behaviour between the two types is exactly similar to that found by KALMUS (1943) between normal and white-eyed members of *Drosophila* species. Both types show phototaxis, but the white-eyed forms do not respond to moving visual contours, as their

ommatidia are not isolated from one another by pigment, and visual acuity is thus reduced to nil. However the light receptors are quite functional when it is irrelevant whether or not they are isolated.

In the larval and pupal stages eye pigment is wholly absent in $\frac{w}{w}$ individuals; nevertheless no difference was found between their behaviour and that of normal individuals. Both showed a well marked shadow response when an opaque object was passed over the water; individuals suspended from the surface film immediately swam downwards. This reaction was also observed when, in a dimly lit room, a bright light placed near a bowl of larvae or pupae was switched off. It was therefore a response to light intensity, not to a moving contour.

*Discussion.* — The main result obtained is that in this species a pair of genes for which both sexes are diploid yet show a partial or incomplete linkage with sex. This phenomenon is not very rare. It is found in *Drosophila* species for the gene *bobbed*, which however at most crosses over very rarely with the sex genes (PHILIP, 1935). It occurs in the beetle *Phytodecta variabilis* (DE ZULUETA, 1925). Here the same sets of genes for natural polymorphism are found both in the *X* and *Y* chromosomes, and crossing over is very rare, if it occurs at all. In this species GALAN (1931) found an unequal pair of chromosomes in the male. So here, as in *Drosophila*, sex is determined by a chromosomal section, but the section of the *X* and *Y* chromosomes which is homologous contains homologous genes.

AIDA (1921) discovered partial sex-linkage in the Cyprinodont teleost *Aplocheilus latipes*, and it has since been found in other Cyprinodonts, notably *Lebistes reticulatus* where WINGE has studied it extensively. He found no unequal chromosomes in the male, nor did any of the numerous sex-linked genes which he found behave like those of *Drosophila* or mammals, where the *Y* chromosome does not carry them. On the contrary WINGE and DITLEVSEN (1938) were able to obtain viable »*YY*», or *MM* individuals provided that the *Y* chromosomes came from different lines. This suggests that the gene for maleness is often coupled with a recessive lethal, but not always with the same one. The fact that either sex may be heterogametic within a group of crossable species, or even within one species (WINGE, 1934), suggests that sex is here determined by genes, and not by chromosome segments.

The only species in which a number of genes showing both types of sex-linkage have been described is man (HALDANE, 1936, 1941).

Here the genes in that part of the *X* which has no homologue in the *Y* show the classical type of sex-linkage, while those which can cross over between the *X* and the homologous segment of the *Y* show partial sex-linkage, detectable in the progeny of heterozygous males as in *Culex*.

The strongest evidence for the determination of sex by a single gene is provided by the work of HUMPHREYS (1945) on the axolotl *Ambystoma mexicanum*. Here the male is normally *ff*, the female *Ff*. By grafting testes which were later removed, HUMPHREYS transformed *Ff* individuals into males. The mating *Ff* ♀ × *Ff* ♂ gave 3 ♀ : 1 ♂, and some of the females gave all female progeny with normal males, and were therefore *FF*. In this species femaleness therefore seems to be due to a single completely dominant gene. It is suggested that where sex is genetically determined in *Pisces*, *Amphibia*, many *Nematocera*, and probably *Reptilia*, it is usually determined by a single gene, even if we accept SVÄRDSON's (1945) presumptive evidence for a chromosome fragment determining sex in *Coregonus lavaretus*, which is not conclusive.

The fact that a worker so experienced as SUTTON in detecting small differences between homologous polytene chromosomes failed to find them in *Culex* suggests that if they exist they are at most of the slight nature associated with some single gene differences in *Drosophila*. An inversion which would allow several genes to act as a unit, or a deficiency or duplication of more than perhaps a single band would probably have been detected. The same applies to *Chironomus* and other Nematoceran species which have been extensively studied. A definite proof of the existence and viability of *MM* cells would strongly support the hypothesis of a single gene. In any case the sex-determining mechanism has as much right to be described as a gene as have such genes as *Bar*, *Delta*, or *Moiré* in *Drosophila melanogaster*.

On the assumption that sex is determined by a gene, or a small chromosomal abnormality which is not lethal when homozygous, and on the further hypothesis that somatic crossing over or abnormal mitosis occurs, we can perhaps explain the occurrence of spanogyny, or shortage of females, which ROUBAUD (1933) and TATE and VINCENT (1936) have observed in *Culex pipiens*. As a result of somatic crossing over mosquitoes or testes of genotype *MM* may arise, and their progeny will be entirely male. Spanandry could also be explained if other genes could determine sex, as they do in *Lebistes*. Lethal genes closely coupled with *m* could produce a shortage of females, but probably not families of 100 ♂ and 1 ♀ as reported by TATE and VINCENT.

Crossing over has so far only been detected in the spermatogenesis of *Culex*. It does not occur, except very rarely at mitosis, in *Drosophila* males. A comparison of its frequency in the sexes of *Culex* must await the discovery of two linked genes.

Although sex determination by a single gene is simpler than by a chromosome, and appears to be primitive in vertebrates, it cannot be assumed to be a primitive character in the *Nematocera*, if only because chromosomal sex determination is found in insect orders more primitive than the *Diptera*. Although the *Nematocera* are more primitive than the *Brachycera*, it is entirely possible that their ancestors had a sex determining mechanism like that of *Drosophila*. For STURTEVANT (1945) describes an autosomal recessive gene, *transformer*, in *Drosophila melanogaster* which has no effect on males, but transforms *XX* or even *XXY* zygotes which would otherwise be females, into males of normal morphology, apart from small size of the testes, and normal behaviour, but sterile. If a similar gene, or the same one with suitable modifiers, produced fertile males, it would be possible to secure a race in which the chromosomes of the sexes would be alike, and femaleness would be due to a single dominant gene.

The gene for white eyes in *Culex* is probably homologous with that in *Drosophila*, where there is only one locus giving this effect. If other sex-linked genes in *Drosophila* have sex-linked homologues in *Culex* it may be possible to homologize the whole or parts of the sex-determining chromosome of *Culex* and the *X* of *Drosophila*. If so this will be an argument for the primitive character of the condition in *Culex*, which could obviously evolve into the *Drosophila* mechanism by a simple process. So long as crossing over occurs between the chromosomes carrying *M* and *m*, or so long as *MM* testes occur with appreciable frequency, the chromosomal regions round the *M* locus are not shielded from natural selection. If, as the result of inversions, suppression of crossing over in the male, and suppression of somatic crossing over, these regions are shielded, recessive lethals, and ultimately deficiencies, will accumulate in them, and the *M* chromosome will degenerate into a nearly functionless *Y*. It is a striking fact that this has not happened in many large and successful groups.

The high variability of crossing over is the more striking since DE WINTON and HALDANE (1935) in *Primula sinensis* and SPURWAY (1945) in *Drosophila subobscura* found that crossing over was hardly more variable than was to be expected as a result of sampling, and decidedly less so than single factor segregation.

Our results emphasize the need for a widespread comparative study of sex-linkage. It is most unlikely that *Homo sapiens* is the only species in which both types of sex-linkage are found for a number of genes; and the partial type found in *Culex* may be far commoner than has been supposed.

We have to thank Professor P. A. BUXTON for allowing this work to proceed in his department, and for his interest in it; Dr. M. J. D. WHITE for photographing the head of a gynandromorph; and Dr. H. SPURWAY for suggesting the hypothesis here put forward as to the origin of the gynandromorphs.

*Summary.* — White eye in *Culex molestus* is a recessive character giving normal Mendelian ratios. It is partially linked with sex, showing $6{,}3 \pm 0{,}6$ % recombination. There are no sex chromosomes, but maleness appears to be due to a single dominant gene in the same chromosome as that for white eye. Crossing over is unusually variable. Three gynandromorphs, two being mosaic for eye colour, are described. These latter cannot be due to chromosomal elimination, and may be due to somatic crossing over.

## LITERATURE CITED.

1. AIDA, T. 1921. On the inheritance of color in a fresh-water fish *Aplocheilus latipes*, TEMMICK and SCHLEGEL, with special reference to sex-linked inheritance. — Genetics 6, 554.
2. BAUER, H. 1935. Der Aufbau der Chromosomen aus den Speicheldrüsen von *Chironomus thummi* KIEFER. — Z. Zellforsch. 23, 280.
3. — 1936. Beiträge zur vergleichenden Morphologie der Speicheldrüsenchromosomen. — Zool. Jb. (Phys.) 56, 239.
4. BEDFORD, G. A. H. 1914. A curious mosquito. — Trans. Roy. Soc. S. Afr. 4, 143.
5. BRELJE, R. v. D. 1923. Ein Fall von Zwitterbildung bei *Aëdes mergenanus*. (*Diptera*: *Culicidae*.) — Arch. mikr. Anat. 100, 317.
6. CLASSEY, E. W. 1942. Gynandromorphism in *Theobaldia annulata* SCHRANK. (*Diptera*: *Culicidae*.) — Entomologist 75, 181.
7. EDWARDS, F. W. 1917. Notes on *Culicidae* with descriptions of new species. — Bull. ent. Res. 7, 201.
8. GALAN, F. 1931. Estudios sobre la espermatogénesis del coleóptero, *Phytodecta variabilis*. — Eos. 7, 461.
9. GOLDSCHMIDT, R. and KATSUKI, K. 1931. Vierte Mitteilung über erblichen Gynandromorphismus und somatische Mosaikbildung bei *Bombyx mori* L. — Biol. Zentbl. 51, 38.
10. HALDANE, J. B. S. 1936. A search for incomplete sex-linkage in man. — Annals of Eugenics 7. I.

11. HALDANE, J. B. S. 1941. The partial sex-linkage of recessive spastic paraplegia. — Journ. Genet. XLI, 2 and 3.
12. HUMPHREYS, R. R. 1945. Sex determination in Ambystomid salamanders. A study of the progeny of females experimentally converted into males. — Am. Journ. Anat. 76, 33.
13. KALMUS, H. 1943. The optomotor responses of some eye mutants of *Drosophila*. — Journ. Genet. 45, 206.
14. MARSHALL, J. F. 1938. The British mosquitoes. — London.
15. MARSHALL, J. F. and STALEY, J. 1937. Some notes regarding the morphological and biological differentiation of *Culex pipiens* LINNAEUS and *Culex molestus* FORSSKÅL. (*Diptera*: *Culicidae*.) — Proc. Roy. Ent. Soc. Lond. (A). 12, 17.
16. MIDDLEKAUFF, W. W. 1945. Gynandromorphism in recently collected mosquitoes. — J. Econ. Ent. 37, 297.
17. PHILIP, U. 1935. Crossing over between the *X* and *Y* chromosomes in *Drosophila melanogaster*. — Journ. Genet. 31, 341.
18. — 1942. An analysis of chromosomal polymorphism in two species of *Chironomus*. — Journ. Genet. 44, 129.
19. ROUBAUD, E. 1933. Assai synthétique sur la vie du moustique commun (*Culex pipiens*). L'évolution humaine et les adaptations biologiques du moustique. — Ann. Sci. nat. Zool. 16, 5.
20. SHUTE, P. G. 1926. Intersexual form of *Ochlerotatus punctor* KIRBY var. *mergenanus*. — Entomologist 59, 12.
21. SMYLY, W. J. P. 1942. A gynandromorph of *Aëdes aegypti* L. (*Stegomyia fasciata*). — Proc. R. Ent. Soc. Lond. (A). 17, 111.
22. SPURWAY, H. 1945. The genetics and cytology of *Drosophila subobscura*. 1. Element A. Sex-linked mutants and their standard order. — Journ. Genet. 46, 268.
23. STURTEVANT, A. H. 1945. A gene in *Drosophila melanogaster* that transforms females into males. — Genetics 30, 297.
24. SUTTON, E. 1942. Salivary gland type chromosomes in mosquitos. — Proc. Nat. Acad. Sci. 28, 268.
25. SVÄRDSON, G. 1945. Chromosome studies in *Salmonidae*. — Reports from the Swedish State Institute of Fresh-Water Fishery Research, 23. Stockholm.
26. TATE, P. and VINCENT, M. 1936. The biology of autogenous and anautogenous races of *Culex pipiens* L. (*Diptera*: *Culicidae*.) — Parasitology 28, 115.
27. WEYER, F. 1938. Ein Zwitter von *Culex pipiens*. — Zool. Anz. 123, 184.
28. WHITE, M. J. D. 1945. Animal cytology and evolution. — Cambridge.
29. WINGE, Ö. 1934. The experimental alteration of sex chromosomes into autosomes and vice versa, as illustrated by *Lebistes*. — C. R. Lab. Carlsberg 21, 1.
30. WINGE, Ö. and DITLEVSEN, E. 1938. A lethal gene in the *Y* chromosome of *Lebistes*. — C. R. Lab. Carlsberg 22, 203.
31. DE WINTON, D. and HALDANE, J. B. S. 1935. The genetics of *Primula sinensis*. III. Linkage in the diploid. — Journ. Genet. 31, 67.
32. WOLF, E. 1941. Die Chromosomen in der Spermatogenese einiger Nematoceren. — Chromosoma 2, 192.
33. DE ZULUETA, A. 1925. La herencia ligada al sexo en el coleoptero *Phytodecta variabilis*. — Eos. 1.

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# EXPERIMENTAL STUDIES ON THE INFLUENCE OF DDT INSECTICIDE UPON PLANT MITOSIS

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## I. INTRODUCTION.

THE c-mitotic activity of colchicine and some other substances has been one of the most central objects of cytological investigation during the last few years. At the same time as new efficient agents have been searched for, the physiology of the c-mitosis has been under careful examination. It may be considered as an undeniable merit of two Swedish investigators (LEVAN and ÖSTERGREN, 1943; ÖSTERGREN and LEVAN, 1943; ÖSTERGREN, 1944 a) that the many different parallel events related to the c-mitosis have been logically connected. The interpretation they give of the physiological mechanism of the c-mitosis will undoubtedly make an established basis for continued elucidation of this question.

The above-mentioned papers have established that in most organic substances the essential character of the c-mitotic activity is physical and therefore does not immediately depend on the chemical structure of a substance. According to LEVAN and ÖSTERGREN (1943) and ÖSTERGREN (1944 a), c-mitosis has many contacts with the narcosis phenomenon and may be considered as a kind of narcosis (in the widest sense of the word). Thus we are able to understand the c-mitotic activity of the most different organic substances in spite of their great variation in efficiency, i. e. the intensity of their visible effect. ÖSTERGREN (1944 a) distinguishes the following closely interrelated effects of organic chemicals on higher plants: *c-mitosis*, which can be *complete* or *partial*, *c-tumour formation*, *chromosome contraction* and many cases of *poison effect*.

It is, however, not necessary that all these related effects are visible at the same time. The active substance may thus be either efficient or inefficient as regards the individual effects. In certain cases other modes of activity can also be observed, though in the current literature there is little to be found on this aspect. These modes may probably

be specific to certain agents in some cases. As a whole, we are as yet very imperfectly acquainted with the above-mentioned phenomena.

When the present writer began to experiment with the DDT insecticide, which during the last few years had proved to be very effective, practical purposes were nearest in his mind. In practice it is often necessary to dust or spray DDT upon the surface of the plant buds at the critical stage of meiosis. Now, it might be thought that a possibly occurring c-mitotic or other effect could give rise to circumstances which would be noticed in practical farming. I have, however, undertaken to experiment with mitoses because, with them, the experiments are simpler to carry out, especially as it has been observed in the few cases studied that the same agents are active in meiosis also. Very probably these investigations will not contribute in any noticeable degree to the practical fight against the insect pests. However, the several interesting features observed, which are unfamiliar in the normal c-mitotic effect, ought to be worth a rather detailed description.

The DDT insecticide is a substance synthesized by the Swiss chemical factory GEIGY (Basle) and as a chemical compound is named dichloro-diphenyl trichloro-methylmethane. DDT is a contact insecticide that is very poorly soluble in water. According to LÄUGER, MARTIN and MÜLLER (1944), the influence of DDT upon the insects depends on its lipoid solubility. Because of this property the substance is able to penetrate the epicuticula and chitin cuticula of the insects and enter into the ends of the nerves. There, it has a strong toxic effect. Its solubility in lipoids is considered to be based upon the chloroform radical, its toxic effect, on the other hand, upon the chlorobenzene part of the DDT molecule.

## II. MATERIAL AND METHODS.

As experimental objects I have used the common commercial varieties of the cultivated onion, *Allium Cepa* ($2n = 16$) and a leguminous plant *Trigonella foenum graecum* ($2n = 16$; cf. FRYER, 1930). The methods have been the same as those successfully used by the Swedish investigators (LEVAN and ÖSTERGREN, 1943) in their experiments with *Allium* and *Pisum*. The capacity of the glass jars in which the objects were growing was 30 cc. The DDT substance used (melting point 106—107° C.) was produced by Dr. T. VORTILA in the laboratory of the State Alcohol Monopoly, Helsinki, Finland. One of the series of the pure DDT substance has been made with the use of saturated tap-

water solutions. In order to bring about an easy dissolving the solutions have been made by pouring a small amount of saturated alcoholic solution of DDT into tap-water and boiling out the alcohol from the aqueous suspension.

Besides these solutions, others containing both ethyl alcohol and DDT have been made in such a way that a definite amount of DDT has been dissolved in a definite amount of absolute alcohol and the solution then poured into tap-water. The solubility of DDT is also quite low in ethyl alcohol. The solutions contain, therefore, rather large amounts of alcohol, i. e. 1, 2, 3 and 4 cc. per 30 cc. water, corresponding to 3,3, 6,7, 10 and 13,3 vol. %. In order to investigate the influence of the pure ethyl alcohol also an alcohol series of corresponding concentration was used.

The root-tips were fixed with twenty-four hours' intervals after 1, 2 and 3 days; in the pure DDT solutions also after 4, 5 and 7 days. The only fixative used was the craf modification according to RANDOLPH.

Exclusively the paraffin method has been used during the investigation and mainly longitudinal sections were made. Staining was partly done by the common iodine-gentian-violet method. As in the course of the examinations it appeared necessary to distinguish between the nucleolus and the chromosomes, recourse was also had to a differential staining. At first I attempted to use the differential staining method of SEMMENS and BHADURI (1939), in which the chromatin is stained by the FEULGEN method and the nucleoli with light-green. However, as the light-green stain was found to be extracted too easily during the differentiation procedure, I tried to combine the common iodine-gentian-violet staining with the FEULGEN method, and the results have been satisfactory.

After being removed from the fuchsine-sulphurous acid and passed through the sulphurous acid water the slides were immediately stained according to the common iodine-gentian-violet method. For the purpose of obtaining the desired tint, the gentian-violet stain must be rather weak in order not to conceal the FEULGEN stain. Therefore, it is best to stain only a short time (2 min. for *Allium* and 5 min. for the less stainable *Trigonella*) and to continue the differentiation in absolute alcohol so long that the FEULGEN stain beneath becomes dimly visible at a macroscopical inspection. The most reliable result can be attained, however, by controlling the differentiation process under the microscope. The chromatin comes out as dark purple, the nucleoli as bluish green. This staining method, however, does not admit of general application, and

the desired result can only be reached by using root-tips treated with ethyl alcohol and DDT + ethyl alcohol. On untreated slides or slides treated only with pure DDT the nucleolus either fails to stain or the stain disappears during the differentiation.

A different gentian-violet staining of euchromatin and heterochromatin at various mitotic stages could clearly be observed. In the euchromatic parts of the resting nuclei the pure red colour of the FEULGEN stain could be seen, while the heterochromites were a very distinct purple. In the cases where the matrix substance was diminished at metaphase the heterochromatic parts of the chromosomes could also be distinguished at these stages.

## III. THE RESULTS.

### 1. ALLIUM CEPA.

*The pure DDT substance.* — As above mentioned, saturated tap-water solutions were used in all experiments. The first fixation was made after twenty-four hours' treatment. After this time of treatment, as well as after all other times up to seven days, no c-mitotic effect except the extra chromosome contraction could be observed. This is on an average 30 % of the total length of the chromosomes. In other respects the divisions were normal.

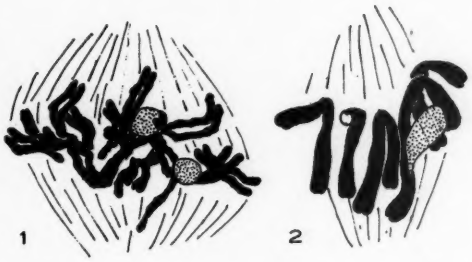
The toxic effect of the DDT substance seems to be weak. The roots were still fully turgescent after treatment lasting seven days. After two weeks, however, the roots seem to be killed.

The pure DDT substance is, thus, a weak mitosis-disturbing agent, the effect of which reaches its maximum in producing the extra chromosome contraction. According to ÖSTERGREN (1944 a, p. 443), this effect is the only visible expression of the c-mitotic properties, which we can see when using the lowest concentrations giving effects in the experiments. The cause of the absence of other c-mitotic effects in the present case — such as are usually brought about by stronger concentrations — is the poor solubility of the DDT substance. This makes it impossible to use sufficiently strong concentrations. In this respect DDT substance can be compared with such substances as diphenyl, azobenzene, dimethylaminobenzene, MICHLER's ketone, phenanthrene and anthracene, with which experiments have been conducted by ÖSTERGREN (l. c.).

The toxicity threshold of the DDT substance also lies obviously

above the water solubility limit in the saturated solutions. In this respect the substance is reminiscent of the likewise very poorly soluble acenaphthene (ÖSTERGREN, l. c.).

*Ethyl alcohol.* — As earlier mentioned, in the pure ethyl alcohol series the concentrations of 3,3—13,3 vol. % were used. In the lowest concentrations the true c-mitotic disturbances affecting spindle function were rarer than in higher ones, nor was the intensity of the other disturbances comparable with that in the strongest solutions. ÖSTERGREN (1944 a, p. 446) mentions the c-mitotic threshold concentration of ethyl alcohol as 1,0—0,5 mol./l, corresponding to the concentration of about 5,8—2,9 vol. %. Thus the results obtained correspond closely with the values published by him. Neither can any macroscopically visible




Figs. 1—2. *Allium Cepa.* — Fig. 1. A metaphase side view of mitosis treated with ethyl alcohol. Two nucleolar bodies The visible structure of the chromosomes is not drawn. — Fig. 2. A part of the metaphasic chromosome set of an untreated plant. The nucleolus attached to a chromosome is visible. — × 1850.

poison effect be observed during the first few days in the highest concentrations.

In the alcohol series an extra chromosome contraction corresponding to that found when the pure DDT substance is used can be observed. The average contraction is also the same, i. e. 30 % of the normal chromosome length.

Besides the chromosome contraction, some features peculiar to the partial c-mitosis have also been observed. As characteristic of these partial c-mitoses the metaphase chromosomes are arranged in the middle of the cell. In several cases a rather complete metaphase orientation may occur, even when the higher alcohol concentrations are used. No achromatic sphere on the surface of which the chromosomes are placed (SHIMAMURA, 1939; BERGER and WITKUS, 1943) can be observed.

No X-shaped metaphase chromosomes characteristic of the metaphase stage of the full c-mitosis can be seen in these partial c-mitoses. The chromosomes are clearly divided into two chromatids lying parallel

to one another during metaphase (Fig. 1). The failure of the centromeres to divide at metaphase of the full c-mitosis has an influence on the elastic repulsion (ÖSTERGREN, 1943) between the two chromatids. Those forces which produce X-shaped chromosomes have not been at work in this case.

Characteristic of the partial c-mitosis is (ÖSTERGREN, 1944 a) the fact that the spindle apparatus is not so much disturbed as to prevent anaphase, but the spindle is unable to give a bipolar anaphase. This is the common case in *Allium* treated with ethyl alcohol. The chromatids separate, sister-nuclei are formed and a cell-wall arises between nuclei. No formation of polyploid cells peculiar to the full c-mitosis can be observed.

In the root-tips treated with alcohol very important changes are observed in the matrix or kalymma surrounding the chromonema, i. e. in the substance containing nucleic acid and basic proteins around the chromonema (CASPERSSON, 1936, and later with collaborators; SERRA, 1942, 1944). Already at prophase, when the accumulation of matrix substance begins in the normal chromosome cycle, we can see that the formation of matrix is weaker than usual. The chromatids are seen to be already separate at an early stage. The spiral structure of the chromonema is quite clearly visible. Lack of matrix substance continues during the whole mitosis. At telophase there then follows the definitive disappearance of matrix belonging to the normal chromosome cycle. The literature seems to contain but few reports of corresponding observations. It is, however, very probable that the same phenomenon can be brought about by other agents too. LEVAN (1945) has observed a corresponding influence from several inorganic salts. BERGER and WITKUS (1943, Plate 1) have published some micro-photographs of c-mitoses induced by colchicine in *Allium*. These show in my opinion a lack of matrix substance similar to that mentioned above. In their experiments with liquid benzene and benzene vapour BERGER (1944) and BERGER, WITKUS and SULLIVAN (1944) have particularly mentioned the phenomenon under consideration.

It can be assumed that the matrix substance of the chromosomes has become more fluid owing to the treatment with ethyl alcohol. This phenomenon is named by RESENDE (1941) »chromatic agglutination». The viscosity of the substance has decreased and it follows that the chromosomes and particularly the distal ends of them have acquired a tendency to glue together, causing the sticky phenomenon. This manifests itself at the anaphase separation of the chromatids when

»false» bridges arise between the two chromatid-sets. In the root-tips treated with alcohol the sticky phenomenon is, however, less pronounced than in those treated with DDT + alcohol, as will be shown later. By no means all anaphases show the sticky phenomenon, and the false bridges are no longer visible after anaphase.

An important feature is also the occurrence of nucleoli even after prophase. This phenomenon, like the stickiness of the chromosomes, is also more intensive after treatment with DDT + alcohol.

The nucleolus behaves during prophase entirely normally, i. e. it diminishes in bulk at the end-stage of prophase so that by the same time the nuclear membrane — or nuclear-cytoplasmic surface, as it has been named by DARLINGTON (1937) — disappears the nucleolus has become very small or has wholly disappeared. Irrespective of that, most of the metaphase figures show nucleoli fastened upon the chromosomes (Fig. 1). The number of nucleoli is usually one or two, more rarely three or four. In the alcohol series the nucleoli disappear wholly at anaphase or at latest after the beginning of telophase, probably being resorbed into the cytoplasm. At telophase no lagging nucleoli between the nuclei could be seen. The formation of the new nucleoli beginning at telophase occurs entirely normally, i. e. the nucleoli begin to appear as a rule at two points inside the group of the telophasic chromosomes. The so formed nucleoli as a rule fuse very soon to one large body.

The fibre structure of the spindle does not in any way deviate from the normal.

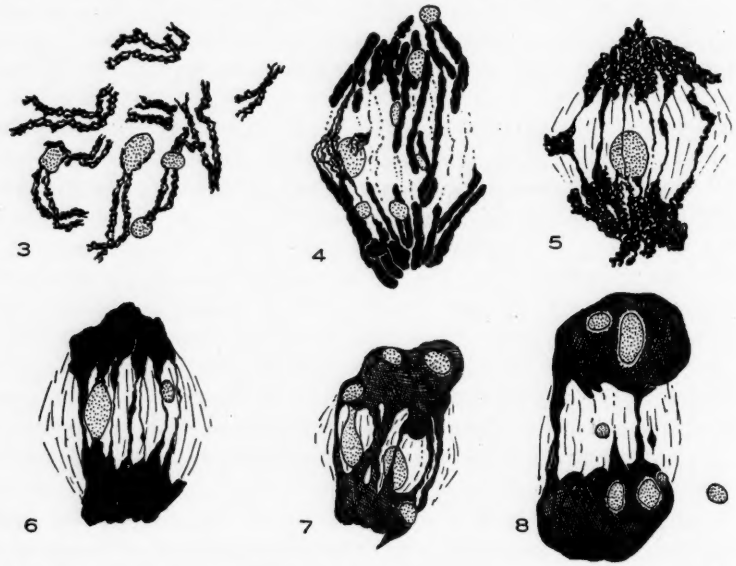
*The DDT substance + ethyl alcohol.* — In the solution series prepared of DDT suspensions of different concentrations the variation in the amount of the suspended substance has no influence. Neither do the different treatment-times — the shortest time was twenty-four hours — cause any essential differences in the quantity or quality of the effect. On the other hand, in the solutions of the lowest alcohol concentration (3,3 vol.%) the c-mitotic effects were weaker and the regular metaphase orientation occurred quite often.

The extra chromosome contraction has always been observable. In amount it corresponded closely to that mentioned above in connection with the pure DDT and the pure ethyl-alcohol treatment. This contraction was on an average 22 % of the normal length of the chromosomes.

At metaphase we can observe the same c-mitotic effect as after alcohol treatment, though more distinctly expressed. The regular metaphase orientation cannot be observed in any case except that of the

lowest alcohol concentration. The chromosomes are always scattered in the middle of the cell (Fig. 3). This figure also gives us a conception of the shape of the chromosomes at this stage. X-shaped chromosomes are entirely lacking. The chromatids lie parallel and they very often seem to be quite separate. They are frequently relationally coiled.

The spindle apparatus is often functional, as can be seen from the typical anaphase and the normal separation of the chromatids. In




Figs. 3—8. *Allium Cepa.* DDT + ethyl alcohol treatment. — Fig. 3. A part of a metaphase plate with scattered chromosomes. Four metaphase nucleoli, lack of the matrix, and the two-partite structure of the chromatids are visible. — Fig. 4. A false bridge and many parts of the metaphase nucleoli fusing with chromatids are visible. A chromatin elimination attached to the largest nucleolus is to be seen. The structure of the chromatids is not drawn. — Fig. 5. A late anaphase with false bridges. — Figs. 6—8. Telophases with numerous false bridges. In Fig. 8 a small chromatin elimination and one metaphase nucleolus pushed out from the division figure are visible. — × 1850.

several cases, however, the occurrence of multipolar spindles can be observed, which feature according to ÖSTERGREN (1944 a) is very characteristic of the partial c-mitosis. Formation of polyploid cells has to some extent occurred. The disturbances have thus in several cases led to full c-mitosis.

The weak development of matrix substance like that mentioned

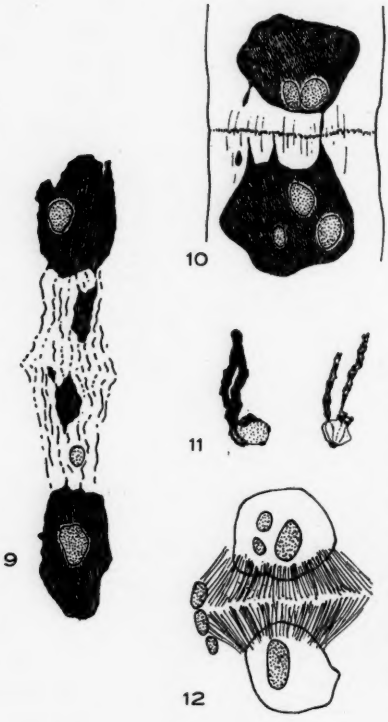
above in connection with the pure alcohol treatment is even more pronounced. At met-anaphase the chromatids seem to be quite »naked» as at the resting stage (Fig. 3) and the chromonemic structure of the chromatids is clearly visible. The two-parted structure of the chromatids is to be indubitably seen (cf. LEVAN, 1945). Whether the half chromatids are further divided, and what the structure of the chromonema is (cf. NEBEL, 1939), has not been examined in this connection.

The »chromatic agglutination» and the sticky phenomenon originating from it are regularly visible after DDT + alcohol treatment both at ana- and telo-phase. The intensity of the stickiness compared with alcohol treatment is also increased. Figs. 4—10 give us a conception of the phenomenon from anaphase to the end stage of telophase. It can be observed that the gluing of the chromatids occurs principally end to end, i. e. the distal ends of the chromatids have the strongest tendency to fuse. In some cases (Fig. 5, the outermost bridge to the left) we can, nevertheless, see that the larger pieces of the chromatids also are able to fuse. The anaphase separation stretches the fused chromatids so that numerous false bridges arise. The durability of the bridges in the DDT + alcohol series is much greater than those after alcohol treatment. They usually last until late telophase (Fig. 8). These bridges rarely occur during the formation of the cell-wall between the sister-nuclei (Fig. 10). This feature seems to be typical of the bridges induced by ÖSTERGREN (1944 b) with ethylene glycol.

The bridges break between the delayed chromosomes and give to the sister-nuclei a very irregular shape. It is impossible to say whether the bridges may sometimes separate intact. Some features, however, suggest that at the break the one suffers deletion while the other gets a duplication. It happens very often that stretching connected with the stickiness breaks the chromatids in such a way that some of their pieces are eliminated. The eliminations visible in Fig. 9 are uncommonly large, in general they are smaller, as can be seen in Figs. 8 and 10. The rôle of the lagging nucleoli in these eliminations must also be mentioned.

The nucleoli appearing after prophase are essentially similar to those found after the alcohol treatment, and their frequency is increased in this case too. In the nucleolar cycle from telophase via resting stage to prophase no deviations from the normal can be observed. Here, too, at the end-stages of prophase the nucleoli are either very insignificant or have wholly disappeared. At the metaphases, however, we can regularly see nucleoli attached to the chromosomes

(Fig. 3). Their number is in general 2—4. In view of the fact that the prophase nucleoli are resorbed in the normal way before the disappearance of the nuclear-cytoplasmic surface *we must consider the metaphasic nucleoli as new formations and not as residues of the foregoing nucleolar cycle.* I have not found in the literature any corresponding case, but RESENDE (1938, p. 393) draws attention to its prob-




Figs. 9—12. *Allium Cepa.* DDT + ethyl alcohol treatment. — Fig. 9. A late telophase with exceptionally large chromatin eliminations. — Fig. 10. The false bridges have persisted until the formation of the cell-wall. Observe the asymmetry of the true nucleoli in the sister-nuclei. — Fig. 11. The formation of the metaphase nucleoli in the chromosomes. In the chromosome to the left the visible chromonema is not drawn. To the right, two fibrils are discernible corresponding to the two chromatids. — Fig. 12. Some lagging nucleoli and the coarsened structure of the spindle are visible. — × 1850.

ability in one case observed by him. The bulk of the nucleoli increases during metaphase and is often rather considerable at anaphase (Figs. 6 and 7). During metaphase we can frequently see that many of the chromosomes are glued to the nucleoli. It may also occur that two nucleoli at metaphase fuse to one larger body. They thus connect the chromosomes producing the nucleoli with other chromosomes from the vicinity.

In contradiction to the conditions after alcohol treatment the nu-

cleoli are not resorbed at anaphase but remain and, in addition to the stickiness, cause a more difficult separation of the chromatids (Figs. 6 and 7). Very frequently during the separation of chromatids the nucleoli break into many pieces and these fasten at haphazard on the chromosomes (Fig. 4). At the end of anaphase, however, the nucleoli are released from the chromosomes and small parts of the anaphase chromosomes follow them in many cases (Fig. 4, the large nucleolus to the left). The loosened nucleoli are resorbed very slowly, lagging at telophase (Figs. 8, 9 and 12). Some of the lagging nucleoli can, however, become enclosed in the arising sister-nuclei (Fig. 8, the smallest nucleolus in the nucleus below). However, that these nucleoli should continue their existence and take part in the forming of the normally functioning nucleoli is improbable. At telophase the nucleolar cycle begins anew, the nucleoli being formed on particular regions of the chromosomes. The symmetry between the nucleoli that have arisen in the sister-nuclei, as observed by HEITZ (1931 a and b) and others at the normal mitoses, has been seriously disturbed (Figs. 8, 10 and 12). This is, however, quite intelligible, as the telophasic polarity is disturbed owing to the abnormal mitosis.

It is difficult to see on which chromosomes and in what manner the metaphase nucleoli arise, as they are connected with other chromosomes as well as those forming them. Still, in favourable cases we can find some elucidating details.

In the normal mitoses of *Allium* we usually find two nucleoli which arise at the SAT-constrictions of the satellited chromosome pair (cf. HEITZ, 1932, p. 597). We can frequently see, however, three nucleoli at the same time. It may thus be assumed that, besides the ordinary SAT-constrictions, also other constrictions take part in normal circumstances in the forming of nucleoli.

It is impossible to identify the nucleolar chromosomes at metaphase, especially as the disappearing of the matrix substance destroys the normal shape of the chromosomes. The number of the metaphase nucleoli (from one to five) indicates that the satellited chromosomes cannot be the only organizers of the nucleoli. On the contrary, it is very possible that the organizers of true nucleoli, the SAT-constrictions, do not at all take part in the forming of the metaphase nucleoli.

In most cases the metaphase nucleoli arise on the distal ends of the chromosomes (Figs. 1, 3 and 11), but occasionally also the intercalary location seems to be possible (Fig. 3, the outermost nucleolus to the right). One may often get the impression that the nucleoli arise as

terminal knobs on the ends of the chromosomes, as DOUTRELIGNE (1933) has stated. The favourable figures show, however, that this is not the case. The nucleolus has arisen subterminally on a zone intercalary to a part of the chromosome much resembling a satellite (Fig. 11). Obviously, this small piece is frequently eliminated with the lagging nucleoli at ana-telophase. The nucleolar substance swells out of its proper place of origin, occasionally even filling long gaps between the chromatids.

In some cases the nucleoli contain very thin fibrils that give the FEULGEN reaction. The chromosome to the right in Fig. 11 represents a case where these are very clearly visible. It could be observed that each chromatid corresponds to two fibrils inside the nucleolar body. The existence of the fibril-like structures in the nucleolar regions of the chromosomes, i. e. the continuation of the chromonema in the heterochromatic region also, has been observed in many cases (cf., e. g., KAUFMANN, 1938). The number of the chromonemata — two per chromatid — corresponds with the observations of GATES and PATHAK (1938) in *Crocus*.

As HEITZ (1932) has already stated, the distal ends of the somatic chromosomes of *Allium* are heterochromatic. In this investigation the same condition has been observed, which seems to occur frequently in the chromosomes of several organisms (cf., e. g., KOSTOFF, 1938 a and b), DARLINGTON (1937) has also pointed out that the distal ends of the chromosomes have a tendency to become heterochromatic and inert, i. e. the telomeres assume a deviating character from the other parts of the chromosomes. *The origin of the metaphase nucleoli may thus be connected with the heterochromatic telomere parts of the chromosomes.*

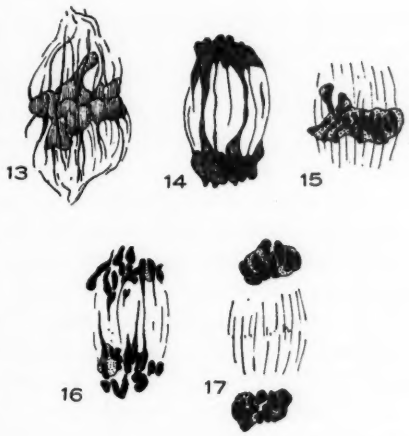
After DDT + alcohol treatment certain changes in the spindle structure have been quite frequently observed. In the slides stained with iodine-gentian-violet a very distinctly stained spindle can be seen (Fig. 12). With FEULGEN staining the structure becomes invisible.

### 2. TRIGONELLA FOENUM GRAECUM.

*Ethyl alcohol.* — Here we have the same alcohol concentrations as with *Allium*. The investigated mitoses are rather normal. It has been impossible to measure the length of the chromosomes, and hence any c-mitotic chromosome contraction that may occur cannot be observed. The formation of metaphase plates is regular. The metaphase chromosomes are to a certain extent more swollen than the normal ones, indicating the presence of nucleolar substance on their surface. Any

stickiness, however, does not occur at anaphase, which is regular. The spindle structure is a little more distinct than normally.

*The DDT substance + ethyl alcohol.* — The same series as with *Allium* have been used excluding, however, the lowest alcohol concentrations, 3,3 and 6,7 vol.%. No true c-mitotic effects can be observed, the chromosome orientation being quite regular. Especially at metaphase in the slides stained with iodine-gentian-violet the chromosomes are, however, swollen and glued together in such a way that the individual chromosomes cannot be distinguished (Fig. 13). The corresponding effect has been observed by ÖSTERGREN (1944 b) in *Allium*




Figs. 13—17. *Trigonella foenum graecum.* DDT + ethyl alcohol treatment. — Fig. 13. A metaphase side view and the coarsened spindle structure. Nucleolar substance covers the whole chromosome set. — Fig. 14. An anaphase with false bridges. Nucleolar substance surrounds the chromosomes. — Fig. 15. A metaphase side view. Nucleolar substance and chromosomes are separately visible. — Fig. 16. An anaphase. The stickiness of the chromosomes and two rather large metaphase nucleoli are to be seen. — Fig. 17. A late telophase. Metaphase nucleoli have disappeared. Two true nucleoli have arisen. — Figs. 13 and 14 are stained with iodine-gentian-violet, Figs. 15—17 with FEULGEN + gentian-violet differential stain. — × 1850.

treated with ethylene glycol. During anaphase the same phenomenon continues, though it is not so conspicuous as at metaphase. Further, at anaphase we can very frequently see stickiness with many false bridges (Fig. 14). The metaphase nucleoli seem, however, to be lacking.

Only the differential staining FEULGEN and gentian-violet revealed that the same conditions as were found in *Allium* are also present in *Trigonella.* It appeared that the swelling and agglutination of the metaphase chromosomes are caused by a nucleolar substance which covers the metaphase chromosomes (Fig. 15). The picture is reminiscent of the cases mentioned by MCCLINTOCK (1934) at the meiosis in *Zea* where the nucleoli arise without nucleolar organizers. As the nucleolar cycle of the ordinary nucleoli of the treated *Trigonella* normally begins

from telophase via the resting stage to the end of prophase, we must regard the metaphasic aggregation of nucleolar substance as corresponding to the metaphase nucleoli in *Allium*.

At the anaphase of *Trigonella* no lagging nucleoli as in *Allium* can be observed. On the contrary, during the anaphase separation the nucleolar substance remains fixed upon the surface of the chromosomes (Fig. 14), disappearing at the same time rapidly. In Fig. 16 we see an anaphase where, of the nucleolar substance, there remain two larger bodies in addition to thin layers on the chromosomes which have been impossible to reproduce on the drawing. During telophase as a rule the nucleolar substance is wholly resorbed. An attraction between the chromatids and nucleolar substance seems to be so much greater in *Trigonella* than in *Allium* that separate rounded nucleolar bodies cannot arise.

At telophase the normal nucleolar cycle begins anew. The nucleolar substance has arisen at two points (Fig. 17), which probably correspond to the satellite chromosome pair of *Trigonella*. In general the two nucleoli fuse very soon together to one larger mass.

For examination of the amount of the matrix substance *Trigonella* is not a suitable object owing to the small size of the chromosomes. Using the FEULGEN stain it has, however, in many cases been possible to observe the chromomeric structure of the chromosomes after DDT + + alcohol treatment. Without treatment this was impossible. This indicates clearly the corresponding disappearance of matrix substance as in *Allium*.

In the *Trigonella* material stained with gentian-violet the thickening of the spindle fibres is somewhat more common and distinct than in *Allium*. The metaphase represented in Fig. 13 reveals the spindle structure very clearly.

### 3. SUMMARY OF THE OBSERVATIONS.

Table 1 gives a summarized account of the frequency and intensity of the different effects both in *Allium* and in *Trigonella*. The frequency has been represented by means of asterisks, three asterisks signifying *regularly*, two asterisks *frequently* and one asterisk *rather rarely*. The different degrees of the intensity are marked with three, two or one cross. In the alcohol and the DDT + alcohol series only the highest alcohol concentrations, i. e. 10 and 13,3 vol.%, have been compared.

The table shows that treatment with pure ethyl alcohol has almost exactly the same effects as that with DDT and ethyl alcohol together.

TABLE 1. *The different effects of ethyl alcohol and DDT upon the mitosis.*

| Effect | *Allium Cepa* | | | *Trigonella foenum graecum* | |
|---|---|---|---|---|---|
| | Pure DDT | Ethyl alcohol | DDT + ethyl alcohol | Ethyl alcohol | DDT + ethyl alcohol |
| Chromosome contraction | ++ | ++ | ++ | ? | ? |
| C-mitosis | − | + | ++ | − | − |
| Disappearing of the matrix substance | − | ++ | +++ | −(?) | ++ |
| Sticky phenomenon at anatelophase | − | ** | *** | − | *** |
| Occurrence of metaphase nucleoli | − | ** / ++ | *** / +++ | *** / + | *** / +++ |
| Occurrence of nucleolar bodies at anaphase | − | * | *** | * | ** |
| Lagging nucleolar bodies at telophase | − | − | *** | − | − |
| Thickening of the spindle structure | − | − | ** | − | *** |

*The DDT substance together with ethyl alcohol causes, however, a very clear increase in the frequency and intensity of these effects.*

Both in *Allium* and in *Trigonella* a significant difference between the nucleolar substance at metaphase and the normal true nucleoli can be observed. The true nucleoli are always clearly vacuolized. In the metaphasic nucleolar bodies, on the contrary, vacuoles have never been observed but these bodies remain entirely homogeneous.

### 4. THE SUBSEQUENT EFFECTS OF THE DDT + ETHYL ALCOHOL TREATMENT.

In order to examine the subsequent effects of the treatment with DDT + ethyl alcohol some onions were placed in pure tap-water after a three-days' treatment. Fixations were made after one and two days.

Slides from the two fixations show the metaphasic disturbances to continue similarly to those found during the treatment. The true c-mitotic disturbances have diminished, i. e. the formation of metaphase figures is quite regular. Metaphase nucleoli, however, arise as intensively as earlier. There is now less stickiness and the amount of matrix sub-

stance is more normal. In the new root-tips developed in water the divisions occur normally.

## IV. DISCUSSION.

Of the effects produced by DDT, ethyl alcohol and, above all, these two substances in combination, we would draw attention especially to three, viz. the lack of matrix substance or kalymma, the stickiness of the chromosomes and the arising of nucleolar substance at metaphase. All these phenomena are known to the cytological literature but, as simultaneous phenomena, they have not been observed earlier. It is, therefore, interesting to examine whether these different effects, which the investigations of the last few years have elucidated more effectively, have any causal connection with one another.

It is clear that a very significant cause of the abnormalities mentioned is the specific effect of the chemical compounds used. We know even a great group of c-mitotic active substances that are not able to effect the phenomena in question. Some preliminary experiments made with *Kniphofia aloides* have, however, demonstrated that the same concentrations of ethyl alcohol and DDT as used in *Allium* and *Trigonella* have no effect. At the same time the c-mitotic disturbances are very weak. According to these observations, we have reason to suppose that there also exist other factors which co-operate with the specific chemical effect.

References to the occurrence of nucleoli at the mitotic division stages after prophase are rather common in the literature. In this connection mention may be made of VAN CAMP (1924), YAMAHA and SINOTO (1925), ZIRKLE (1928), DERMEN (1933), SATO (1936), RESENDE (1937), RAMANUJAM (1938) and HÅKANSSON and LEVAN (1942). Corresponding meiotic nucleoli have been observed by, e. g., DERMEN (1933) and FRANKEL (1937). The occurrence of the nucleoli has, however, been described as a more or less sporadic phenomenon. In some cases, as for instance according to LEVAN (HÅKANSSON and LEVAN, l. c., p. 439) in Leguminosae plants, it can be very common. RAMANUJAM (l. c.) has noticed the phenomenon quite regularly at mitoses of *Oryza*. Some of these observers state that the nucleoli persist from the foregoing prophase. RESENDE (1938) only reports the possibility that in the case published by him (1937) it might be a question of a new nucleolus. The regularity and the precocity in the occurrence of the metaphase nucleoli were thus previously unknown phenomena.

On account of the classical investigations of HEITZ (1931 a and b)

it has become clear that the normal nucleoli are formed by definite parts of the chromosomes, i. e. the SAT-constrictions. This fact has been verified so often that in spite of some contradictory observations (e. g., DERMEN, 1933; DOUTRELIGNE, 1933) it cannot be questioned. The investigations of MCCLINTOCK (1934) have to a great extent elucidated the function of these nucleolar regions. Above all it has been made clear that the activity of the nucleolar regions is genically controlled. RESENDE (1938) has criticized the interpretations of MCCLINTOCK, and he ignores the nucleolar-substance-producing function of the nucleolar regions. He thinks these to be nucleolar-substance-collecting organs in which the true nucleolus arises from material called by him pre-nucleolar substance. The occurrence of this depends on the existence of heterochromatin in the cell.

HEITZ (1931 a), by establishing the lack of nucleic acid in the nucleolar region and naming them SAT- (Sine Acido Thymonucleinico-) constrictions, has really verified the heterochromacity of them. Since then the relation between heterochromatin and nucleoli has been still more clearly expressed (cf. SERRA, 1942; HUSKINS, 1942; STRAUB, 1943). Cytology has at last escaped the obscurity and the speculations (cf. DERMEN's review, 1933, on the subject) which have dominated the question of the function of nucleolus. Especially the investigations of CASPERSSON (1940, and later with collaborators) have made it possible to establish a hypothesis on the probable mode of function of the nucleolus.

DARLINGTON and LA COUR's (1938, 1940) observations on the so-called »differential segments» have shed new light on the problem of heterochromatin. According to them, these are heterochromatic regions which do not become visible at normal metaphases but after cold treatment can be seen as unstained zones in several parts of the chromosomes. This so-called negative heterochromacy (KLINGSTEDT, 1941) is caused by a certain »starvation» of nucleic acid of the heterochromatin. The other investigators have also observed a similar »allocycly» of heterochromatin in plants (GEITLER, 1940 a; TSCHERMAK, 1943) as well as in animals (KLINGSTEDT, 1941; WICKBOM, 1945). The proximate cause of allocycly in the case represented by KLINGSTEDT was the hybridity of the animals.

It is, however, as yet unexplained what the relation is, on one hand, between the above mentioned differential segments and the nucleolar regions and, on the other hand, between the last-mentioned and the non-SAT-constrictions (RESENDE, 1938) which take no part in

the formation of the nucleoli. LEVAN (1942) thinks that secondary constrictions in the haploid rye observed by him correspond to the differential segments. KLINGSTEDT (1941) has in an interesting way dealt with this question. As a difference between euchromatin and heterochromatin he mentions the timing difference shown by the nucleic-acid cycle of the two substances. The nucleic acid of euchromatin disappears after telophase, becoming again distinctly visible at next prophase. In this way the nucleic-acid cycle of heterochromatin is behind — or perhaps rather before — the cycle of euchromatin. The disappearance of the nucleic acid occurs so near the beginning of the new synthesis of nucleic acid that the achromatic phase practically does not exist. External agents, e. g. low temperature, can cause a delay in the synthesis of new nucleic acid and thus affect the visibility of the differential segments at this period. KLINGSTEDT (l. c., p. 173) mentions that great similarity to the nucleolar regions, and says: »One may perhaps tentatively suggest that the nucleolus organizer is a special kind of heterochromite, the productive activity of which is postponed until telophase and consists in a condensation of chromosome products so much changed as not to show the staining reactions of thymonucleic acid any more».

DARLINGTON (1941) has observed, in some *Paris* species, the occurrence of differential segments to be genically controlled. In some cases such conditions exist that allocycly cannot appear simultaneously with the nucleolar regions. He mentions, it is true, also the existence of both phenomena at the same time. GEITLER (1940 a), TSCHERMAK (1943) and WICKBOM (1945) have also observed the same case in several organisms.

Though it is clear that heterochromatin forms a very heterogeneous group of substances, it seems obvious that *we can find several functional similarities between nucleolar regions* (= *SAT-constrictions*), *non-SAT-constrictions and differential segments.* They are all heterochromites, the nucleic-acid cycle of each being specially differentiated. The capability to form nucleoli may also be similar. If we want to widen the above-mentioned hypothesis of KLINGSTEDT we must draw out the conclusion that the nucleolar regions and non-SAT-constrictions are heterochromites having an allocycly that is permanently changed. The nucleolar regions are differentiated as places where the true nucleoli as a rule arise. The non-SAT-constrictions may also be able to form nucleoli. That this may often occur can be seen from the fact that the number of nucleoli is greater than that of SAT-constrictions (cf.

DERMEN, 1933). Differential segments, on the contrary, cannot as a rule be distinguished owing to failure of the necessary intervening time in their nucleic-acid cycle. Their heterochromatin is, however, as usual (cf. SERRA, 1942) very sensitive to changes of the external and internal factors. In this case the sensibility may be even greater than usual. The cold treatment (DARLINGTON and LA COUR, 1940; GEITLER, 1940 a) or the unbalanced genotype due to hybridisation (KLINGSTEDT, 1941) can, for instance, cause the arising of differential segments.

It is probable that there are many other modifying factors the character of which is as yet unknown. It has, for instance, been impossible to distinguish between non-SAT-constrictions and differential segments in the investigations on the chromosome morphology. This is confirmed by the observations of the variation of secondary constrictions during the various stages of mitosis. SVÄRDSON (1945) has observed that in several *Salmo* species the number of constrictions is greatest at prophase and after that rapidly diminishes so that the constrictions are no longer visible and stain normally. This supports, as SVÄRDSON (l. c., p. 36) has also suggested, the above mentioned hypothesis of KLINGSTEDT concerning the character of allocycly. The morphological characters of constrictions can vary even during the same mitosis (CALLAN, 1942; cited after SVÄRDSON, 1945).

It has not been possible to find in the literature observations on the capability of differential segments to take part in the formation of nucleoli. *It seems, however, very probable that many, possibly all, heterochromites contain, at least as potential, the capability to give rise to nucleolar substance.* It would, therefore, be difficult to assume that the differential segment would make an exception.

References to chromosome bridges at anaphase not caused by dicentric chromatids (DARLINGTON, 1937) can be found quite frequently (see literature in TISCHLER, 1942, and DARLINGTON and UPCOTT, 1941). These bridges, which seem to be caused by the sticking together of parts of chromatids, have been named pseudo-pontes by RESENDE (1941), who suggested their cause to be chromatic agglutination. The best known term is, however, sticky phenomenon.

As the cause of stickiness BEADLE (1932, 1937) mentions a special gene (*st*) in *Zea*, KLINGSTEDT (1939) the hybridisation in grasshoppers, SVÄRDSON (1945) the same cause in Salmonids and LÖVE (1944) in *Rumex*. Among the chemical agents WITKUS and BERGER (1944) mention veratrine, ÖSTERGREN (1944 b) ethylene glycol, and LEVAN (1945) several inorganic salts. As other factors WHITE (1937) has observed

the X-ray radiation in grasshoppers and RESENDE (1941) in an *Aloë* hybrid, COUTINHO (1940; cited after RESENDE, 1941) a virus in *Vicia faba*. No external factors at all seem, according to RESENDE (l. c.), to be implicated in the sticky phenomenon in *Aloë mitriformis* var. *Commelinii*.

A statement of special interest is that of DARLINGTON and LA COUR (1940) concerning the coincidence of sticky phenomenon with differential segments. GEITLER (1940 b), CALLAN (1942) and WICKBOM (1945) comment on a similar tendency in their objects. The stickiness of the chromosomes may, therefore, in these cases be considered as caused by changes in the heterochromatin. KLINGSTEDT (1939) has explained the phenomenon as caused by a delay in the lapsing of the attraction of the sister-chromatids as compared with the onset of the anaphase movement. This is in accordance with the interpretation of DARLINGTON and LA COUR (1940) that the sticking tendency of the differential segments is due to a hindrance in the development of genes caused by nucleic acid starvation. The unreduplicated chromatids are, as a consequence, not able to separate. Later, however, DARLINGTON and UPCOTT (1941) have advanced another interpretation, viz. that the stickiness is due to the reunion of chromatids broken at earlier stages owing to cold treatment.

If against this background we consider the disturbances caused by ethyl alcohol and DDT + ethyl alcohol, we can observe that they are confined to metaphase. The other division stages including the resting stages are quite normal. As was stated earlier, the met-anaphasic abnormalities are: the appearance of metaphase nucleoli, the sticky phenomenon, the lack of matrix substance, and the spindle apparatus disturbance characteristic of c-mitosis. Among these partial effects the formation of metaphase nucleoli and sticky phenomenon has been suggested to be connected with the function of heterochromatin. The harmony in the collaboration of spindle apparatus and centromeres has a great significance for the continuance of the timing balance of mitosis. DARLINGTON (1937, p. 547) has expressed this thus: »The formation of the spindle and the successful orientation and division of the chromosomes therefore depend on a balance in the strength and timing of the cycles and repulsion of the centromeres and centrosomes». The disturbances in the normal timing relationships between several stages of division are very significant causes of division abnormalities. KLINGSTEDT (1939, p. 411) emphasizes the importance of them in his statement concerning DARLINGTON's precocity theory: »Without going

into the discussion which is still going on about the precocity theory as such, I would call attention to the general type of interpretation of which the precocity theory is a special case: I mean changes in the timing relationships of events, which play an important part in DARLINGTON's theories and will no doubt prove a valuable method of interpretation in biology».

When we examine the metaphasic abnormalities in *Allium* and *Trigonella* it is not possible to escape the impression that the disturbances just in the timing relationships are one of the most significant causes of them. *The abnormalities are strikingly reminiscent of conditions which, normally, are peculiar to telophase and resting stage.*

The diminishing of matrix substance is a process normally beginning at telophase and attaining its maximum at resting stage. In *Allium*, on which the most careful observations were possible, the lack of matrix substance already begins at prophase. At metaphase the chromosomes have the same external structure as at the end stages of telophase or resting stage, excluding the persisting spiral. Using the FEULGEN gentian-violet differential staining it is possible, just as at resting stage, to distinguish in the metaphase chromosomes the heterochromites as dark stained segments from the euchromatic parts. The observations of KOSTOFF (1938) on the metaphasic heterochromites in *Crepis capillaris* and *Triticum monococcum* are very similar. There is no allocycly of heterochromatin here. The clearness of the spiral structure is exclusively due to the absence of the matrix that usually covers it. It cannot be analogous to the straightening of the chromonema spiral on treatment with several chemical agents, because the extra chromosome contraction exists simultaneously. The chromosomes of *Trigonella* have not been so successful objects for corresponding observations, but, nevertheless, it may be assumed that the same conditions prevail.

The formation of nucleoli is also a phenomenon normally occurring at telophase. It seems that in *Allium* as well as in a corresponding way in *Trigonella* the true nucleolar regions do not take any part in the forming of the metaphase nucleoli. These do not begin formal function until at telophase. It is clear that the heterochromatic segments of *Allium*, especially in the telomeric parts, begin to form nucleolar substance exceptionally and at exceptional time. That supports the earlier suggestion that not only the SAT-constrictions but also the many other heterochromites have a potential and in definite cases manifested capability to form nucleolar substance. A peculiar feature in this case

is the precocity of this manifestation compared with the normal nucleolar cycle.

The conditions observed in *Trigonella* deviate from those in *Allium*, the nucleolar substance not being formed as different bodies but entirely surrounding the metaphasic chromosome set. This is reminiscent of the meiosis of *Zea* described by McCLINTOCK (1934), where the nucleolus has arisen without any special nucleolar regions. On this ground one might conclude that *Trigonella*, except SAT-constrictions inactive at this stage, has no differentiated heterochromites, as *Allium*, capable of forming nucleoli at metaphase. The nucleolar substance might thus arise along the surface of the chromosomes. DERMEN (1933) considers this mode of developing as normal. Another and, as I think, a more possible alternative is that the nucleolus-forming capacity of *Trigonella* is relatively greater than that of *Allium* (the normal nucleolus—nucleus ratio is in *Trigonella* greater than in *Allium*). The substance formed on the heterochromites, therefore, quickly fills the spaces between the small chromosomes.

Metaphase nucleoli disappear before telophase in the two examined species. In *Allium* these often lag a long time, in *Trigonella* they are quickly dissolved in the cytoplasm. In both cases the nucleoli are not able to function further. As earlier mentioned, the metaphase nucleoli are never vacuolized. DERMEN (1933) has especially pointed out the significance of vacuolization as a criterion of functioning nucleolus. Because the nucleolar regions at telophase begin to form new, functioning nucleoli it has been suggested that *the metaphase nucleoli are not true nucleoli.* They are built up from a substance which probably would be identified with matter called by HEITZ (1931 a) n-substance and by RESENDE (1938) pre-nucleolar substance. This is raw material from which only the SAT-constrictions are able to form true nucleoli. Heterochromites accidentally producing pre-nucleolar substance have no such capability. It is important, however, to pay attention to the wholly different environment in which the metaphase nucleoli and true nucleoli have arisen. It seems conceivable that *the arising of true nucleoli presupposes a phase in which the nuclear sap surrounds the SAT-constrictions.*

The cause of the sticky phenomenon, the strong attraction of the distal heterochromites, indicates conditions prevailing at telophase and above all at the resting stage. As KLINGSTEDT (1941) has remarked, at the stages mentioned above heterochromites have a tendency to fuse and form bodies resembling nucleoli. For this reason it has been

suggested that *the sticky phenomenon is caused by precocity in the development of the heterochromites.* These have fused similarly as at resting stage, and we can show that the cause of attraction is the purely mechanical agglutination, as RESENDE (1941) also has pointed out. The interpretation given by DARLINGTON and LA COUR (1940) that the duplication of the chromonema is hindered by the lack of nucleic acid cannot be valid here because no starvation of nucleic acid has been observed. The later interpretation of DARLINGTON and UPCOTT (1941) also seems to be inappropriate, for it is difficult to assume a necessary number of chromatid breaks at earlier stages.

The synchronous disturbance of timing relationships at many stages of chromosome division is the chief results of the treatment described above. Abnormalities occur in the euchromatic as well as in the heterochromatic parts of the chromosomes. Metaphase chromosomes are actually under development at a stage corresponding to the telophase and resting stage. *As a real cause of the disturbance of timing relationships we must no doubt consider the c-mitotic disturbance of the spindle apparatus.* The chromosomes are in such a condition that the heterochromatic segments, but not the SAT-constrictions, begin to produce nucleolar substance. The substance formed constitutes the raw material of true nucleoli, so-called pre-nucleolar substance, and is later eliminated. The beginning of true nucleolus development presupposes that the SAT-constrictions are surrounded by nuclear sap. As a direct result of the precocity there also arises the sticky phenomenon with false bridges, although undoubtedly the agglutination of matrix caused by the used chemicals is also a very important factor.

Owing to the fact that the stickiness and simultaneously occurring metaphase nucleoli prevent the anaphase separation there are frequent breaks of the chromatids. It has been possible to observe this directly at anaphase of *Allium.* Especially the parts of chromatids fused with nucleoli are liable to become entirely eliminated. Since the poison effect of DDT + ethyl alcohol is relatively weak, the question should be investigated whether the effects at meiosis correspond to those observed at mitosis. BEADLE (1932, 1937) has observed a strong increase of the mutation rate in the progeny of maize plants affected by stickiness of the chromosomes.

It is an interesting feature, though not one that is possible to explain without further investigation, that the ethyl alcohol strengthens the effects of DDT. Are the described abnormalities the result of the co-action of the two chemical compounds? It seems, however, im-

probable that both these substances could have a similar specific effect. It is rather possible that only ethyl alcohol is likely to possess the property in question, DDT playing no direct part. Experiments with ethyl alcohol in combination with several other c-mitotically active substances could probably shed light on this problem.

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In this connection I wish to extend my sincere gratitude to the Director of the State Horticultural Institute (Piikkiö, Finland), Prof. Dr. O. MEURMAN, for help and valuable criticism during my work in his institute. I am also very grateful to Mag. phil. EEVA SUOMALAINEN for her appreciated assistance. I am greatly indebted to Dr. A. LEVAN for valuable advice and fertile discussions during my stay at the Cytogenetic Laboratory of Svalöf Plant-Breeding Institute in the summer of 1945 and for all the ready help later given in the course of my work. I also thank Dr. G. ÖSTERGREN, who has read the manuscript and made valuable suggestions. In addition I wish to thank the Chemist of the Finnish Alcohol Monopoly, Dr. T. VOITILA, for the DDT substance kindly produced by him and Dr. V. KANERVO for supplying me with the literature concerning DDT.

## SUMMARY.

(1) An investigation is submitted of the effects of treating the root-tips of *Allium Cepa* and *Trigonella foenum graecum* with DDT insecticide (dichloro-diphenyl trichloro-methylmethane), ethyl alcohol and DDT + ethyl alcohol.

(2) The combined FEULGEN and iodine-gentian-violet differential stain was used to distinguish nucleolus and chromatin. In objects treated with ethyl alcohol and DDT + ethyl alcohol this gave a satisfactory result.

(3) Pure DDT substance (saturated tap-water solutions) was observed to have a weak c-mitotic effect. The extra contraction of metanaphasic chromosomes was measured.

(4) In *Allium Cepa* treatment with pure ethyl alcohol (3,3—13,3 vol.% tap-water solutions) caused, besides the extra chromosome contraction, the effects characteristic of partial c-mitosis. It was observed that, in the heterochromatic telomere parts, metaphase chromosomes produce nucleolar bodies which are dissolved in the plasm during anaphase. The matrix around the chromonemata is weakly developed.

The structure of the chromonema is quite clearly visible. At anaphase the sticky phenomenon can be seen, i. e. the tendency of the distal ends of chromosomes to fuse and thus form false bridges.

The proper nucleolar regions of the chromosomes, SAT-constrictions, do not take part in the formation of metaphase nucleoli but function normally. Hence the cycle of the true nucleoli is regular. The metaphase nucleoli are not residues of prophasic true nucleoli but are new formations that have arisen after prophase.

In *Trigonella* a small amount of nucleolar substance is produced on the surface of the chromosomes by treatment with ethyl alcohol. Sticky phenomenon does not occur. Metaphase figures are normal.

(5) Using DDT + ethyl alcohol (saturated DDT solution + 3,3—13,3 vol.% ethyl alcohol), the same effects as with ethyl alcohol series become visible, though they are more powerful and frequent.

In *Allium*, besides the above mentioned c-mitotic chromosome contraction, multipolar spindles and in some cases polyploid cells also occur. Metaphase nucleoli are not wholly resorbed in the plasm but remain as laggards between the sister-nuclei. Chromatid breaks and small chromatin eliminations also take place owing to the stickiness and the hindering effect of the lagging nucleoli on the anaphase separation.

In *Trigonella* the metaphasic nucleolar substance entirely covers the chromosomes. This substance coating the chromosomes does not give rise to lagging nucleolar bodies. The substance disappeared before the beginning of telophase. Sticky phenomenon is intensive. Metaphase orientation seems to be regular.

(6) When the onions treated with DDT + ethyl alcohol are transferred to pure tap-water, even after two days the same mitotic abnormalities as in root-tips fixed at the DDT + alcohol treatment can be observed.

(7) The current literature concerning the development of the above-mentioned mitosis abnormalities has been studied. The conclusion can be drawn that functional similarities can be found between the SAT-constrictions, non-SAT-constrictions and the heterochromatic regions of chromosomes called differential segments. These heterochromites have, if not manifestly yet at all events potentially, the capability of forming nucleolar substance.

(8) It is suggested that, besides the specific effect of ethyl alcohol and DDT, the disturbance of timing relationships between the different stages of the mitotic chromosome cycle is one of the most important

causes of the described mitotic abnormalities. The disturbance is caused by the c-mitotic narcosis of the spindle apparatus and the consequently retarded division. Euchromatic as well as heterochromatic parts of chromosomes are at metaphase already in a developing phase corresponding to telophase and resting stage. In normal conditions the disturbances or the direct consequences of them are characteristic phenomena of the above-mentioned stages.

(9) It has also been suggested that the metaphase nucleoli consist of pre-nucleolar substance, i. e. of the raw material of true nucleoli, and are not, therefore, able to function. In the author's opinion the true nucleoli cannot as a rule arise elsewhere than in connection with SAT-constrictions when these are surrounded by nuclear sap. The true nucleoli cannot here be formed from this pre-nucleolar substance which is thus excluded from the normal nucleolar cycle.

## LITERATURE CITED.


1. BEADLE, G. W. 1932. A gene for sticky chromosomes in *Zea mays*. — Zschr. f. ind. Abst.- u. Vererbungsl. 63: 195—217.
2. — 1937. Chromosome aberration and gene mutation in sticky chromosome plants of *Zea mays*. — Cytologia, FUJII Jub. Vol.: 43—56.
3. BERGER, C. A. 1944. Experimental studies on the cytology of *Allium*. — Torreya 44: 41.
4. BERGER, C. A. and WITKUS, E. R. 1943. A cytological study of c-mitosis in the polysomatic plant *Spinacia oleracea*, with comparative observations on *Allium cepa*. — Bull. Torr. Bot. Club 70: 457—467.
5. BERGER, C. A., WITKUS, E. R. and SULLIVAN, B. J. 1944. The cytological effect of benzene vapor. — Bull. Torr. Bot. Club 71: 620—623.
6. CALLAN, H. G. 1942. Heterochromatin in *Triton*. — Proc. Roy. Soc. London. Ser. B. 130: 324—335.
7. VAN CAMP, G. M. 1924. Le rôle du nucléole dans la caryocinèse somatique (*Clivia miniata* REG.). — Cellule 34: 1—50.
8. CASPERSSON, T. 1936. Über den chemischen Aufbau der Strukturen des Zellkerns. — Skand. Arch. f. Physiol. 73, Suppl. 8: 1—151.
9. — 1940. Die Eiweissverteilung in den Strukturen des Zellkerns. — Chromosoma 1: 562—604.
10. COUTINHO, L. DE A. 1940. Os virus como agentes modificadores dos chromosomas. — Rev. Agron. 28: 83.
11. DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd ed. — London.
12. — 1941. Polyploidy, crossing-over and heterochromatin in *Paris*. — Ann. of Bot. N. S. 5: 203—216.
13. DARLINGTON, C. D. and LA COUR, L. 1938. Differential reactivity of the chromosomes. — Ann. of Bot. N. S. 2: 615—625.

14. DARLINGTON, C. D. and LA COUR, L. 1940. Nucleic acid starvation of chromosomes in *Trillium*. — Journ. of Genetics 40: 185—213.
15. DARLINGTON, C. D. and UPCOTT, M. B. 1941. Spontaneous chromosome change. — Journ. of Genetics 41: 297—338.
16. DERMEN, H. 1933. Origin and behavior of the nucleolus in plants. — Journ. Arn. Arbor. 14: 282—323.
17. DOUTRELIGNE, J. 1933. Chromosomes et nucléoles dans les noyaux du type euchromocentrique. — Cellule 42: 31—100.
18. FRANKEL, O. H. 1937. The nucleolar cycle in some species of *Fritillaria*. — Cytologia 8: 37—47.
19. FRYER, J. R. 1930. Cytological studies in *Medicago*, *Melilotus* and *Trigonella*. — Can. Journ. Res. 3: 3—50.
20. GATES, R. R. 1937. The discovery of the relation between nucleolus and the chromosomes. — Cytologia, FUJII Jub. Vol. 979—986.
21. GATES, R. R. and PATHAK, N. G. 1938. Chromosome structure. — Nature 142: 156—157.
22. GEITLER, L. 1940 a. Neue Ergebnisse und Probleme auf dem Gebiet des Chromosomenbaues. — Naturwissensch. 28: 649—656.
23. — 1940 b. Temperaturbedingte Ausbildung von Specialsegmenten an Chromosomenenden. — Chromosoma 1: 554—561.
24. HEITZ, E. 1931 a. Die Ursache der gesetzmässigen Zahl, Lage, Form und Grösse pflanzlicher Nucleolen. — Planta 12: 774—844.
25. — 1931 b. Nucleolen und Chromosomen in der Gattung *Vicia*. — Planta 15: 495—505.
26. — 1932. Die Herkunft der Chromocentren. — Planta 18: 571—636.
27. HUSKINS, C. L. 1942. Structural differentiation of the nucleus. The structure of protoplasm. — Ames, Iowa: 109—126.
28. HÅKANSSON, A. and LEVAN, A. 1942. Nucleolar conditions in *Pisum*. — Hereditas XXVIII: 436—440.
29. KAUFMANN, B. P. 1938. Nucleolus-organizing regions in salivary gland chromosomes of *Drosophila melanogaster*. — Zschr. f. Zellf. u. mikr. Anat. 28: 1—10.
30. KLINGSTEDT, H. 1939. Taxonomic and cytological studies on grasshopper hybrids. I. Morphology and spermatogenesis of *Chorthippus bicolor* CHARP. × *Ch. biguttulus* L. — Journ. of Genetics 37: 389—420.
31. — 1941. Negative heterochromacy in Orthopteran chromosomes. — Mem. Soc. pro Fauna et Flora Fenn. 17: 166—175.
32. KOSTOFF, D. 1938 a. A contribution to the chromosome structure and behavior. — Cellule 47: 219—255.
33. — 1938 b. Heterochromatin at the distal ends of the chromosomes in *Triticum monococcum*. — Nature 141: 690.
34. LEVAN, A. 1938. The effect of colchicine on root mitoses in *Allium*. — Hereditas XXIV: 471—486.
35. — 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII: 177—211.
36. — 1945. Cytological reactions induced by inorganic salt solutions. — Nature 156: 751—752.

37. LEVAN, A. and ÖSTERGREN, G. 1943. The mechanism of c-mitotic action. — Hereditas XXIX: 381—443.

38. LÄUGER, P., MARTIN, H. and MÜLLER, P. 1944. Über Konstitution und toxische Wirkung von natürlichen und neuen synthetischen insektentötenden Stoffen. — Acta Chim. Helvetica 27: 892—928.

39. LÖVE, A. 1944. Cytogenetic studies on *Rumex* subgenus *Acetosella*. — Hereditas XXX: 1—156.

40. MCCLINTOCK, B. 1934. The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. — Zschr. f. Zellf. u. mikr. Anat. 21: 294—328.

41. NEBEL, B. R. 1939. Chromosome structure. — Bot. Rev. 5: 563—626.

42. RAMANUJAM, S. 1938. Chromosome studies in the Oryzeae. — Ann. of Bot. N. S. 2: 107—125.

43. RESENDE, F. 1937. Über die Ubiquität der SAT-Chromosomen bei den Blütenpflanzen. — Planta 26: 757—807.

44. — 1938. Nucleoli and SAT-chromosomes. — Bol. da Soc. Broteriana. 2. Ser. 13: 391—424.

45. — 1940. Über die Chromosomenstruktur in der Mitose der Wurzelspitzen. II. SAT-Differenzierungen, Spiralbau und Chromonemata. — Chromosoma 1: 486—520.

46. — 1941. Movimento, aglutinacao, pontes e distensao dos chromosomas na mitose. — Bol. da Soc. Broteriana. 2. Ser. 15: 163—196.

47. SATO, D. 1936. Chromosome studies in *Scilla*. III. SAT-chromosomes and the karyotype analysis in *Scilla* and other genera. — Cytologia 7: 521—529.

48. SEMMENS, C. S. and BHADURI, P. N. 1939. A technic for differential staining of nucleoli and chromosomes. — Stain Technol. 14: 1—5.

49. SERRA, J. A. 1942. Relations entre la chimie et la morphologie nucléaire. — Bol. da Soc. Broteriana. 2. Ser. 16: 83—135.

50. — 1944. An attempt at a synthesis of the physiological and cytological concepts of the gene. — Bol. da Soc. Broteriana. 2. Ser. 19: 327—369.

51. SHIMAMURA, T. 1939. Cytological studies of polyploidy induced by colchicine. — Cytologia 9: 486—494.

52. STRAUB, J. 1943. Chromosomenstruktur. — Naturwissensch. 31: 97—108.

53. SVÄRDSON, G. 1945. Chromosome studies on Salmonidae. — Rep. Swedish State Inst. of Fresh-Water Fishery Res. 23: 1—151.

54. TISCHLER, G. 1942. Allgemeine Pflanzenkaryologie. Bd. II. 2. Hefte. Lief. 1. Kernteilung und Kernverschmelzung. Handb. d. Pflanzenanat. — Berlin—Zehlendorf.

55. TSCHERMAK, E. 1943. Vergleichende und experimentelle cytologische Untersuchungen an der Gattung *Oedogonium*. — Chromosoma 2: 493—518.

56. WHITE, M. J. D. 1937. The effect of X-rays on the first meiotic division in three species of Orthoptera. — Proc. Roy. Soc. London. Ser. B. 124: 183—196.

57. WICKBOM, T. 1945. Cytological studies on Dipnoi, Urodela, Anura, and *Emys*. — Hereditas XXXI: 241—346.

58. WITKUS, E. R. and BERGER, C. A. 1944. Veratrine, a new polyploidy inducing agent. — Journ. of Heredity 35: 129—133.

59. YAMAHA, G. and SINOTO, Y. 1925. On the behavior of the nucleolus in the somatic mitosis of higher plants, with microchemical notes. — Bot. Mag. Tokyo 39: 205—219.
60. ZIRKLE, C. 1928. Nucleolus in root-tip mitosis in *Zea mays*. — Bot. Gaz. 84: 402—418.
61. ÖSTERGREN, G. 1943. Elastic chromosome repulsions. — Hereditas XXIX: 444—450.
62. — 1944 a. Colchicine mitosis, chromosome contraction, narcosis and protein chain folding. — Hereditas XXX: 429—467.
63. — 1944 b. An efficient chemical for the induction of sticky chromosomes. — Hereditas XXX: 213—216.
64. ÖSTERGREN, G. and LEVAN, A. 1943. The connection between c-mitotic activity and water solubility in some monocyclic compounds. — Hereditas XXIX: 496—498.

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# INVESTIGATIONS ON C-MITOSIS IN ALLIUM CEPA

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## I. THE CYTOLOGICAL EFFECT OF HEXACHLORO-CYCLOHEXANE.

SINCE the discovery of the well-known cytological effect of colchicine many other organic compounds have been shown to have the same effect on mitosis. Among other investigators, LEVAN and ÖSTERGREN (1943) and ÖSTERGREN and LEVAN (1943) found c-mitotical activity in about 30 substances, and in 1944 ÖSTERGREN tested about 50 other substances, most of which proved to possess the same properties. The works of LEVAN and ÖSTERGREN also led to the discovery of the principle that the activity of many c-mitotic agents is conditioned not so much by their chemical properties as especially by their physical ones, and this in turn caused them to propound their theory of the narcotic nature of the c-mitosis.

It seemed interesting to us to study the ability of the well-known insecticide hexachlorocyclohexane to induce c-mitosis. Four different isomers of the substance are known, and they are all well separated as regards both their physical properties and their insecticidal effects (cf. SLADE, 1945 a and b). Three pure isomers have been available to us, viz. the $\alpha$-, $\beta$- and $\gamma$-isomers, as also the crude product »666». The composition of »666» is as follows: $\alpha$-isomer 10—12 %, $\beta$-isomer 70 %, $\gamma$-isomer 5 %, $\delta$-isomer 10 %, together with some chlorinated compounds of an unknown nature.

The investigations have been carried out at the Cyto-genetic Laboratory, Svalöf, and at the Institute of Genetics, Lund. Our thanks are due to the Chief of the former Institute, Dr. A. LEVAN, as also to Dr. G. ÖSTERGREN, Lund, for their valuable advice and for discussing the problems. We are also very much indebted to A.-B. Pharmacia (Mr. E. ASKELÖF), Stockholm, and A.-B. Astra (Dr. S. WIEDLING), Södertälje, for their ready courtesy in providing us with the substances needed.

## 1. METHODS.

In the main the methods used were the same as those employed earlier by LEVAN and ÖSTERGREN (1943). To get exact concentrations of the substances we first made concentrated alcohol solutions, which were then diluted with water (tap-water). The strongest concentration of all isomers made in this way contained 0,07 % ethyl alcohol, which, however, could hardly have had any effect of interest in our case. As material for the treatments we used bulbs of *Allium Cepa*, the roots of which were immersed in the various solutions. The root tips were fixed in Navashin and, after embedding, cut into longitudinal sections and stained with crystal violet.

## 2. DESCRIPTION OF THE RESULTS.

At the study of the cytological effects there appeared some very interesting differences between the isomers. The results of the treatments are collected in Table 1. Beginning with the c-mitosis, the most insecticidal $\gamma$-isomer proved to be able to induce complete c-mitosis in concentrations of 0,000·01 mol/litre and more. In 0,000·005 mol/litre there occurred multipolar anaphases indicating spindle disturbances, partial c-mitosis. The complete c-mitosis was quite typical and agreed with that described, e. g., by LEVAN (1938). On the other hand, we never observed any tendencies to such a distribution of the chromosomes as is obtained after treatment with methylnaphthoquinone.

The next most insecticidal $\alpha$-isomer also brought about spindle disturbances, partial c-mitosis, but was unable to induce complete c-mitosis even in saturated solutions. The c-mitotical efficiency of this isomer was thus much weaker than that of the $\gamma$-isomer.

Concerning the third, the least insecticidal $\beta$-isomer, it proved to be quite without any c-mitotical activity, even saturated solutions being unable to produce any disturbances.

The mixture of the various isomers, »666», induced complete c-mitosis, probably owing to its content of $\gamma$-isomer, an assumption supported by the situation of the threshold value. The $\delta$-isomer, not tested by us, may have had some effect as well.

In tests on the ability of the saturated solutions to induce c-tumours the $\gamma$-isomer was also found to differ from the other isomers, being alone able to produce this effect.

No poison effect was obtained in any solutions after 24 hours' treatment, but after 48 hours the roots were quite slack and obviously

TABLE 1. *The various properties of the isomers of hexachlorocyclohexane and the crude product »666».*

+ = presence and — = absence of the effect in question.

| Substances: / The effects: | | $\gamma$ | $\alpha$ | $\beta$ | »666« |
|---|---|---|---|---|---|
| C-mitosis after 4 hours Conc. in mol/litre | 0,000·1 | + | ± | — | + |
| | 0,000·05 | + | ± | — | + |
| | 0,000·02 | + | ± | — | ± |
| | 0,000·01 | + | ± | — | — |
| | 0,000·005 | ± | — | — | — |
| | 0,000·002 | — | — | — | — |
| | 0,000·001 | — | — | — | — |
| C-tumours after 24 hours Saturated solutions | | + | — | — | + |
| Poison effect (death of roots) Saturated solutions | | | | | |
| After 24 hours | | — | — | — | — |
| After 48 — 200 hours | | — | — | — | + |
| Rel. insecticidity *Meligethes* (frequency of dead beetles in % of that of the $\gamma$ -isomer) | | 100 | 94,3 | 70,7 | 87,9 |
| Rel. insecticidity *Colandria* (after SLADE, low value = high insect.) | | 1 | 900 | inactive | |
| Melting point (after SLADE) | | 112,5° | 158° | 309° | |
| Water solubility in $10^{-6}$ mol/litre | | 100— —200 | 50— —100 | 20— —50 | 100— —200 |

dead in saturated solution of »666». The $\gamma$-isomer, although very poisonous to insects, showed no signs of such toxic effects as killing of the roots even after long and repeated treatments with saturated solutions.

To get acquainted with the insecticidal effects of the various isomers of hexachlorocyclohexane we performed some experiments to this end, using the same concentrations of the isomers as used for the c-mitotical treatments.

Turnip blossom beetles (*Meligethes aeneus* F.) were put into covered Petri dishes at the bottoms of which there were filter papers moistened with 1 c. c. of the solutions. After three hours the beetles were counted, and the percentage of dead beetles was determined. These values are represented in Diagram 1. In the main our results coincide with earlier results of other authors. The $\gamma$-isomer proved to be the most insecticidal one, $\alpha$- the next, and finally the $\beta$-isomer the least insecticidal one. The effect of »666» was intermediate.

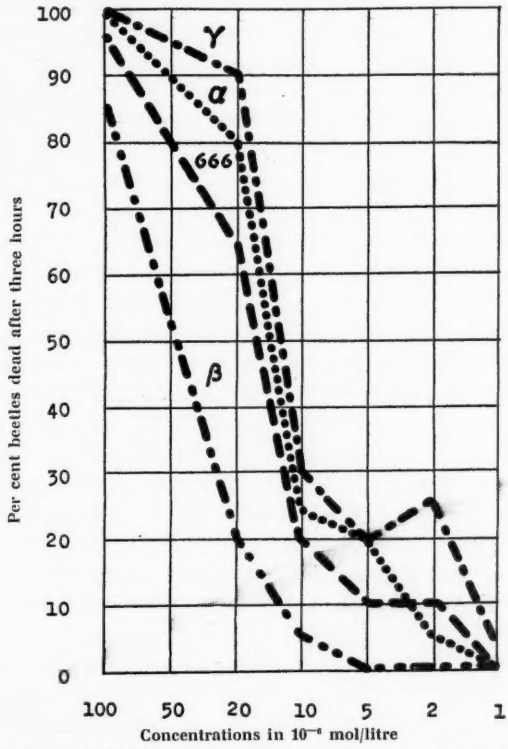



Diagram 1. The insecticidal effects of the various isomers of hexachlorocyclohexane and the crude product »666». Concerning data, see text.

## 3. DISCUSSION.

Insecticides may perform their action in three ways, viz. as contact agents, as fumigants, or as stomach poisons. LÄUGER, MARTIN and

MÜLLER (1944) consider lipoid solubility to be conditional upon the action as contact agent. The cuticle of the insects, among other things, contains lipoids by which the contact agent is supposed to penetrate. A fumigant insecticide must be volatile, and the physical properties of the stomach poisons, finally, may be widely varying.

While certain insecticides, as e. g. the well-known DDT, have a specific contact activity, »gammexane» (the $\gamma$-isomer of hexachlorocyclohexane) by SLADE (1945) is said to be active in all the three ways. Judging from the investigations of SYLVÉN (1947), its action as a fumigant seems to be very important, and the experiences of SLADE point in the same direction.

As regards the mode of action of hexachlorocyclohexane in a physiological respect, it is close at hand to assume that it is acting as a narcotic. Unfortunately no literature on this aspect of the action of gammexane is available to us. Other chlorohydrocarbons, as e. g. chloroform and carbon tetrachloride, being also insecticides, are known as narcotics, and they probably produce their insecticidal effects as narcotics. As is well known, too strong doses of narcotics have a toxic effect, killing the cells instead of producing a reversible narcosis.

According to the classical MEYER-OVERTON theory of narcosis, the narcotics produce their action by dissolving in the lipoids of the cells, and lipoid solubility is usually considered as an important condition of narcotic activity.

If we consider the physical and insecticidal properties of the different isomers of hexachlorocyclohexane, we shall find a connection between low melting point, high water solubility and insecticidity (cf. Table 1). The differences in melting point should undoubtedly also be connected with differences in solubility in most solvents, among them also lipoids, viz. in such a way that low melting point entails a high solubility.

If the insecticidity of these substances is conditioned by their narcotic properties, a connection was to be expected between this insecticidity and the lipoid solubility of the substances. Owing to its relatively low melting point and undoubtedly relatively high lipoid solubility, the $\gamma$-isomer should be expected to have the strongest insecticidal effect.

The rather small differences between the isomers in our insecticidity trial are probably referable to our methods; among other things the gas concentrations were very high in our covered Petri dishes.

Concerning the mechanism of the c-mitotic action, there are in the

main two theories. In 1943 LEVAN and ÖSTERGREN for the mechanism of the c-mitosis proposed a kind of lipoid theory corresponding to the MEYER-OVERTON theory of narcosis and, thus, »the decisive concentration of the substance may be that in the lipoids not in the water phase of the cells». This assumption explains the connection between the solubility and c-mitotical activity which these authors had found to be characteristic of the substances studied. Furthermore, they supposed that the c-mitosis was simply to be considered as a narcosis of certain enzymic functions of the cells. Even the c-tumour effect was considered as a narcosis of the growth control of the cells.

In 1944 ÖSTERGREN had arrived at a somewhat modified theory. He lays stress upon the view that the c-mitosis is to be considered simply as a narcotized cell division. The spindle breakdown characteristic of the c-mitosis he supposes to be caused by the fibrous components of the spindle being brought to assume a more or less corpuscular shape by the action of the c-mitotic agent, and this in turn would lead to the disorganization of the spindle.

Both these theories agree with the connection that seems to exist between the c-mitotic activity of the substances and their solubility relations, viz. a positive correlation between activity and lipoid solubility and a negative correlation between activity and water solubility.

If we put these considerations into connection with the results of our experiments, we shall find that the differences between the isomers may be quite explicable. The $\gamma$-isomer has a relatively low melting point and certainly also relatively high lipoid solubility. Its higher c-mitotic activity and its ability to induce c-tumours are, therefore, completely expected, as was also its insecticidal effects. The $\alpha$-isomer has a higher melting point and lower solubility, and its concentration in the lipoids may be supposed to be just at the threshold of c-mitosis and under that of c-tumours. The $\beta$-isomer, finally, has the highest melting point and the lowest solubility and is for that reason quite without any c-mitotical activity.

As already mentioned, we could not get the $\delta$-isomer. As its melting point (138—139° C.) is between those of the $\gamma$- and $\alpha$-isomers, it might be expected to possess a certain degree of c-mitotic activity.

The differences found by us between the different isomers of hexachlorocyclohexane are completely analogous to those found by LEVAN and ÖSTERGREN (1943) to exist between the $\alpha$- and $\beta$-naphthalene derivatives. The $\alpha$-derivatives studied were all able to induce c-mitosis, whereas the corresponding $\beta$-derivatives were characterized by higher

melting points, lower solubility as also by a weaker c-mitotic efficiency.

Regarding the poison effect on the *Allium* roots, its nature sometimes seems to be more unclear, even if it, according to ÖSTERGREN (1944), in many cases may be a narcotic effect too, in such cases probably in some way analogous to the insecticidal effects of certain narcotics. In other cases the poison effect is certainly of another nature than narcotic, especially when it is brought about by substances with high water solubility and low lipoid solubility.

As seen from Table 1, the pure isomers of hexachlorocyclohexane are quite without any poison effects and did not kill the *Allium* roots, whereas saturated solutions of »666» killed the roots after 48 hours' treatment. The poison effect of this crude product is probably caused by some impurities in this substance. We also made some orientating studies with an ether extract of a commercial mixture containing »gammexane». It proved to be able to induce c-mitosis, but it also had a poison effect similar to that of »666», and even in this case probably caused by impurities, e. g., chlorinated decomposition products.

According to LEVAN and ÖSTERGREN (1943), the practical applicability of a substance for the production of polyploids is largely dependent on its lack of toxic properties and its range of active concentrations, i. e. the relation between its solubility and threshold value.

Next to colchicine, which is unique in several respects, acenaphthene is the substance that is responsible for the greatest number of chemically induced polyploids, this depending not only on the fact that it has been in use for a long time, but also that it has proved not to be poisonous.

It does not seem improbable to us that even the $\gamma$-hexachlorocyclohexane might be useful for this purpose. In similarity to acenaphthene it is not poisonous, but in addition its range of active concentrations is much wider. The relation between solubility and threshold concentration is for $\gamma$-hexachlorocyclohexane ca. 30, whereas the same value for acenaphthene is only ca. 4. Judging from our experiences, however, purity seems to be necessary to get rid of the poison effect.

*Summary.* — Three pure isomers and a crude product of the well-known insecticide hexachlorocyclohexane have been studied as regards their cytological effects on *Allium* roots, especially their ability to induce c-mitosis.

The crude product as well as the most insecticidal $\gamma$-isomer proved to be able to induce complete c-mitosis, whereas the $\alpha$-isomer only

induced partial c-mitosis and the $\beta$-isomer was quite without any c-mitotical properties.

There may be a connection between insecticidity, c-mitotical activity and the simple physical properties of the substances, which is in complete agreement with the theory of the narcotic nature of c-mitosis.

As the $\gamma$-isomer was quite without any toxic effects on the *Allium* roots and at the same time was able to induce c-mitosis, it might be practically applicable for the production of polyploids.

## II. THE CYTOLOGICAL EFFECT OF VITAMIN K.

When studying the cytological effect of various organic compounds we investigated, among other things, the ability of three synthetical types of vitamin K to induce c-mitosis.

The substances used were: 2-methylnaphthoquinone, 2-methyl 1—4-naphthohydroquinone diacetate and sodium 2-methylnaphthohydroquinone 1—4-diphosphate. In spite of their simpler chemical constitution these substances have the same medico-biological effect as natural vitamin K, viz. to enable the coagulation of blood by coöperating at the formation of prothrombin (cf. MÖLLER, 1943).

### 1. METHODS.

The methods were the same as used when studying the cytological effect of hexachlorocyclohexane. As material for the treatments we used bulbs of *Allium Cepa*, the roots of which were immersed in the various solutions of the substances.

The methyl-naphthohydroquinone diacetate and sodium-methyl-naphthohydroquinone diphosphate were pure products from A. B. Astra. Under the name of »K-vimin» these substances have pharmacological application, the acetate as an oil solution and the phosphate as pills.

### 2. DESCRIPTION OF THE RESULTS.

*Methylnaphthoquinone.* — The results of the treatments are collected in Table 2. The solubility of the substance seems to be about $0{,}000{\cdot}5$ mol/litre, and it is, thus, to be considered as only very sparingly soluble in water.

In concentrations of $0{,}000{\cdot}1$ mol/litre and more we obtained typical c-mitoses after four hours' treatment. C-tumours did not appear, obviously due to the short time of treatment. The highest concentrations had a very marked poison effect resulting in pycnotic, degenerating

TABLE 2. *The cytological effects of vitamin K.*

+ = presence and — = absence of the effect in question; 0 means that a possibly existing effect may be hidden by poison effect. The first sign of each column refers to four hours' treatment and the second one to 24 hours.

| | 2-methylnaphthoquinone | | | | | | 2-methyl 1—4-naphtho-hydroquinone diacetate | | | | | | Sodium 2-methylnaphtho-hydroquinone 1—4-diphosphate | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration in $10^{-6}$ mol | Precipitate | C-mitosis | C-tumours | Agglutination | Poison effect | Turgescence | Precipitate | C-mitosis | C-tumours | Agglutination | Poison effect | Turgescence | Precipitate | C-mitosis | C-tumours | Agglutination | Poison effect | Turgescence | Concentration in $10^{-6}$ mol |
| 0,1 | | | | | | | | | | | | | — | — 0 | — 0 | ± 0 | — + | + — | 0,1 |
| 0,05 | | | | | | | | | | | | | — | — 0 | — 0 | (±) 0 | — + | + — | 0,05 |
| 0,02 | | | | | | | | | | | | | — | — — | — — | — — | — — | + + | 0,02 |
| 0,01 | | | | | | | | | | | | | — | — — | — — | — — | — — | + + | 0,01 |
| 0,005 | | | | | | | | | | | | | — | — — | — — | — — | — — | + + | 0,005 |
| 0,002 | | | | | | | + | ± 0 | — 0 | — 0 | — + | + ± | | | | | | | 0,002 |
| 0,001 | + | 0 0 | 0 0 | 0 0 | + + | ± — | + | (±) 0 | — 0 | — 0 | — ± | + ± | | | | | | | 0,001 |
| 0,000·5 | ± | + 0 | — ± | + 0 | ± + | ± — | — | — — | — — | — — | — — | + + | | | | | | | 0,000·5 |
| 0,000·2 | — | + + | — + | + — | (±) ± | + ± | — | — — | — — | — — | — — | + + | | | | | | | 0,000·2 |
| 0,000·1 | — | + — | — — | ± — | — ± | + + | — | — — | — — | — — | — — | + + | | | | | | | 0,000·1 |
| 0,000·05 | — | — — | — — | — — | — — | + + | — | — — | — — | — — | — — | + + | | | | | | | 0,000·05 |
| 0,000·02 | — | — — | — — | — — | — — | + + | | | | | | | | | | | | | 0,000·02 |

nuclei, and even in 0,000·2 molar solution there occurred some pycnotic nuclei, especially in the outer cell layers. Consequently, the roots in the two strongest concentrations showed reduced turgescence.

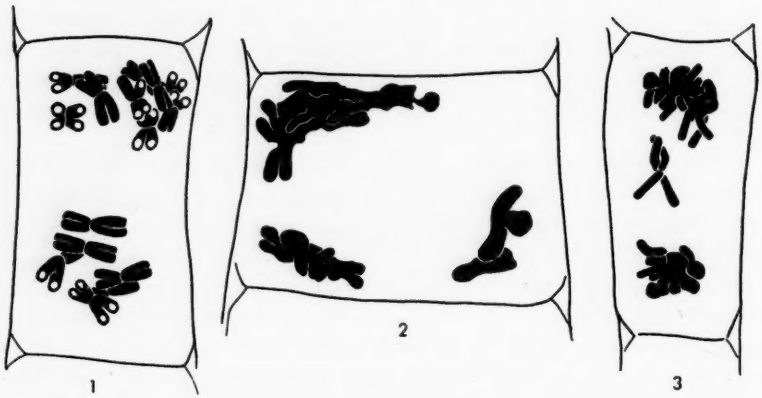
Probably the agglutination of the chromosomes appearing in the same solutions is to be considered as a kind of poison effect too. It expresses itself in the chromosomes becoming liquefied and baked together to an irregular lump. Such agglutination is certainly identical with the »sticky chromosome» effect, several cases of which are known, e. g., after treatment with some other c-mitotically active substances, e. g. ethylene glycol (ÖSTERGREN, 1944). In our case, however, the formation of anaphase bridges due to stickiness is rendered impossible by the lack of spindle activity. In ethylene glycol the threshold concentration of agglutination and stickiness is lower than that of spindle destruction.

After 24 hours' treatment there occurred c-tumours in those of the strongest concentrations in which the toxic action did not hinder the production of this effect. Now the poison effect itself was very marked also in weaker solutions, and even in 0,000·1-molar solution the roots were partly dead, resulting in lack or reduction of turgescence. While the threshold of poison effect had thus been lowered, the threshold of agglutination and c-mitosis had been raised about one step in the concentration series, and these effects were now obtained only in solutions that were at the same time more or less poisonous. The c-mitosis was obtained only in 0,000·2-molar solution. This c-mitosis differed, however, strikingly from the typical one described, e. g., by LEVAN (1938) and SHIMAMURA (1938). Instead of lying scattered in the cells, dividing and forming telophase nuclei, usually one per cell, the undivided chromosomes (c-pairs) are in this case of ours distributed at random to the two poles of the cells, where they divide and are usually inclosed in two telophase nuclei.

At first sight this telophase is similar to a normal telophase, but it differs from this in several respects conditioned by the absence of a normal spindle mechanism in these cells, the distribution of the c-pairs being brought about by some other forces. Firstly, the distribution of the chromosomes is obviously quite at random, sometimes resulting in the formation of »daughter» nuclei of very unequal size. Furthermore, these nuclei are without that polarization of centromeres and chromosome arms typical of normal telophase nuclei, and, finally, they are not separated by a cell-wall, this defect being also caused by the lack of spindle.

When this type of c-mitosis, which we term »*distributed c-mitosis*», is strongly pronounced, the c-pairs seem to be pressed against the end-walls of the cells by some force from its centre. Sometimes there occur not only two but several groups of chromosomes, these lying more or less close to the cell-walls, and the telophase picture is then very much like the »exploded metaphase» described by BARBER and CALLAN (1943).

Occasionally there may also lie a distinct group in the centre of the cells in addition to the polar ones.




Figs. 1—3. Distributed c-mitoses at anaphase and transitions to telophase.

Besides this distributed c-mitosis, there also occur quite typical ones, without distribution of the chromosomes, as also transitions between them, but the type described is much too predominating to be merely a fortuitous phenomenon. No tendencies to such distribution of the chromosomes were seen in any other concentration or after only four hours' treatment.

The chromosomes were contracted to about half their normal length, and their shape was the same as at typical c-mitosis. The late division of the centromeres was very characteristic. Neither spindle fibres in the plasm nor any other signs of spindle activity could be detected.

*Methyl-naphthohydroquinone diacetate.* — The water solubility of this substance too is rather low, 0,001—0,000·5 mol/litre. It proved to be unable to induce complete c-mitosis even in saturated solutions. These solutions, however, possessed a certain degree of c-mitotical activity that

gave rise to multipolar mitoses, regarded as partial c-mitoses by ÖSTERGREN (1944).

After 24 hours' treatment the saturated solutions showed a strong poison effect. No c-tumours were obtained in any solutions.

*Sodium-methylnaphthohydroquinone diphosphate.* — As might be expected from its chemical constitution, the water solubility of this substance is very high. We did not determine the solubility exactly, but even a 1-molar solution was without a precipitate. As seen from the table, this substance is quite devoid of any c-mitotical activity. Not even a 0,1-molar solution was able to bring about any c-mitotical disturbances. The only effects obtained were poison effects after 24 hours' treatment, and tendencies to agglutination of the chromosomes, but these effects probably do not belong to the c-mitotical complex of reactions.

## 3. DISCUSSION.

As to the differences in c-mitotical activity between the substances tested, we would suggest the following explanation.

The works on c-mitosis by LEVAN and ÖSTERGREN undoubtedly show that in many cases the activity of the c-mitotical agents is mainly conditioned by their physical properties, especially their solubility relations. These authors have found that there is a negative correlation between water solubility and c-mitotical activity, and this correlation is explained on the assumption that the substances produce their action dissolved in the lipoids of the cells, and thus the lipoid solubility is of decisive importance.

The sodium-methylnaphthohydroquinone diphosphate is a salt and should therefore hardly be perceptibly soluble in lipoids. Its c-mitotical inactivity is, thus, very well expected. It can never produce its action in the same way as the methylnaphthoquinone, which must be assumed to have at least a certain degree of lipoid solubility and to be able to induce c-mitosis. Concerning the acetate, this substance may also be expected to be somewhat soluble in lipoids and should consequently also be expected to give c-mitosis. It is difficult, however, at present to say whether it should be more or less active than the methylnaphthoquinone, the chemical structure being too different.

It has been more difficult to find a plausible explanation of the origin of the »distributed c-mitosis». Judging from their figures and description, WITKUS and BERGER (1944) seem to have obtained a most similar or even identical deviation from the typical course of c-mitosis

in their »pseudo-anaphase». These authors are of the opinion that there is an imperfect spindle active, but they do not mention anything as to the nature of this spindle. Besides, the term »pseudo-anaphase» is perhaps somewhat inadequate, as typical c-chromosomes also pass through an anaphase and a telophase.

Above it was mentioned that the distributed c-mitosis is sometimes very similar to the »exploded metaphase» obtained by BARBER and CALLAN (1943) in *Triton* after treatment with cold and colchicine. These authors explain its origin in the following way: »In the exploded anaphase the chromosomes are pushed bodily about the cell, probably owing to the formation of a purely centrosome spindle.» The centromeres are considered to be inactive and the c-pairs are pushed about the cell just as at our distributed c-mitosis.

Making use of ÖSTERGREN's (1945) theory concerning the existence of certain transverse forces in the spindle tactoid which act on the chromosome bodies, we are venturing to put forward an explanation of the origin of our distributed c-mitosis, one that is perhaps also applicable to the »pseudo-anaphase» and the »exploded metaphase».

It does not seem impossible to us that the forces acting on the c-pairs might be of the same kind as those proposed by ÖSTERGREN, in normal cells »manifested as tendencies of the chromatids to move out of the spindle» and resulting in the origin of »transverse equilibria on the spindle». It may be suggested that in spite of the absence of any visible spindle mechanism there is still a kind of coacervate or tactoid, the particles of which push the chromosomes in a peripheral direction, thus causing the characteristic distribution of the chromosomes described by us.

In fact, observations by other authors indicate that at c-mitosis there exists an element in the cells that might produce such forces, viz. the achromatic sphere observed by SHIMAMURA (1938) and BERGER and WITKUS (1943) around which the chromosomes sometimes seem to arrange themselves.

The increased threshold concentration of c-mitosis after 24 hours' treatment is also very difficult to interpret. Possibly the cells have a certain ability of getting inured to the treatment. Regarding the camphor reaction of yeast, LEVAN and SANDWALL (1943) state that »the cells may gradually become insusceptible to the substances» during the treatment, whereas ÖSTERGREN (1944) in *Allium* has in some cases observed a »downward movement of the c-mitotic threshold».

*Summary.* — Three pharmacologically used types of vitamin K are tested as regards their ability of inducing c-mitosis in root-tip cells of *Allium*. Methyl-naphthoquinone proved to be able to induce complete c-mitosis, whereas methyl-naphthohydroquinone diacetate only induced partial c-mitosis and sodium-methylnaphthohydroquinone diphosphate was quite without any c-mitotical activity.

The differences in activity agree with the opinion of LEVAN and ÖSTERGREN that the solubility properties are of decisive importance for the c-mitotical activity of organic compounds.

There is also described a peculiar type of c-mitosis that deviates from the typical c-mitosis by having the undivided c-pairs distributed to the two poles of the cells.

An explanation of the origin of this »distributed c-mitosis» is submitted.

## LITERATURE CITED.

1. BARBER, H. N. and CALLAN, H. G. 1943. The effects of cold and colchicine on mitosis in newt. — Proc. Roy. Soc. B. 131: 258—270.
2. BERGER, C. A. and WITKUS, E. R. 1943. A cytological study of c-mitosis in the polysomatic plant *Spinacia oleracea*, with comparative observations on *Allium cepa*. — Bull. Torrey Bot. Club. 70: 457—467.
3. LEVAN, A. 1938. The effect of colchicine on root mitoses in *Allium*. — Hereditas XXIV: 471—486.
4. LEVAN, A. and SANDWALL, C. G. 1943. Quantitative investigations on the reaction of yeast to certain biologically active substances. — Hereditas XXIX: 164—178.
5. LEVAN, A. and ÖSTERGREN, G. 1943. The mechanism of c-mitotic action. — Hereditas XXIX: 381—443.
6. LÄUGER, P., MARTIN, H. and MÜLLER, P. 1944. Über Konstitution und toxische Wirkung von natürlichen und neuen synthetischen insektentötenden Stoffen. — Helvetica Chim. Act. 27: 892—928.
7. MÖLLER, K. O. 1943. Farmakologi. 2. Udg. — Copenhagen.
8. SHIMAMURA, T. 1938. Cytological studies of polyploidy induced by colchicine. — Cytologia 9: 486—494.
9. SLADE, R. E. 1945 a. A new British insecticide, the gamma isomer of benzene hexachloride. — Chem. Trade Journ. and Chem. Engineer 1945: 279—281.
10. — 1945 b. Specific poisons. — Endeavour 1945: 150—153.
11. SYLVÉN, E. 1947. Undersökningar över gammaflyet, *Phytometra gamma* L. — Medd. fr. Statens Växtskyddsanstalt 1947.
12. WITKUS, E. R. and BERGER, C. A. 1944. Veratrine, a new polyploidy inducing agent. — Journ. of Heredity 35: 131—133.

13. ÖSTERGREN, G. 1944 a. An efficient chemical for the induction of sticky chromosomes. — Hereditas XXX: 213—216.

14. — 1944 b. Colchicine mitosis, chromosome contraction, narcosis and protein chain folding. — Hereditas XXX: 429—467.

15. — 1945. Transverse equilibria on the spindle. — Botaniska Notiser, Lund. 1945: 467—468.

16. ÖSTERGREN, G. and LEVAN, A. 1943. The connection between c-mitotic activity and water solubility in some monocyclic compounds. — Hereditas XXIX: 496—498.

# CONTRIBUTIONS TO A CYTOLOGICAL ANALYSIS OF THE SPECIES DIFFERENCES OF GODETIA AMOENA AND G. WHITNEYI

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THE parallel genetical and cytological investigations of the *Godetia* material in the cultures of Dr. HIORTH have given certain results that also seem to be of interest to students of the problem of speciation. Such a result was the production of a *G. Whitneyi* with the new basic number 6, previously unknown in the genus *Godetia* (HIORTH, 1946 c; HÅKANSSON, 1946). The new form had been produced through intraspecific crosses between different monosomics. In this paper the behaviour of the chromosomes in repeated backcrosses of the interspecific hybrid *G. amoena* × *G. Whitneyi* to *G. Whitneyi* will be described, and also some cases of spontaneous chromosome changes.

HIORTH (1942) has shown that *G. amoena* occurs only in a rather small area south of Golden Gate, while *G. Whitneyi* has a wider distribution from Golden Gate to the Vancouver Island in British Columbia. The most important morphological species difference consists of certain patterns on the petals. *G. amoena* has a basal spot quite at the base of the petal; such a spot never occurs in *Whitneyi*. *G. Whitneyi* has a central spot of varying size nearer the centre of the petal; this spot occurs in some garden races of *amoena* but never in *amoena* plants from natural localities. Individuals lacking petal spots may occur in the two species. HIORTH (1940) has shown that the spotless condition is recessive (*f*), that there are several central spot alleles, each causing a different size of this spot ($F^x$), and that the basal spot gene of the *amoena* race used in the crosses and designated $F^b$ is allelic to $F^x$ and *f*. This allelism of $F^b$ and $F^x$ has been fully confirmed by the results of later crosses of HIORTH. Its manifestation is perhaps unusual; $F^bF^x$ plants have both a basal and a central spot. Earlier, RASMUSON (1921) had studied the genetics of these petal spots and found that »a case of multiple allelomorphism possibly is present here» (l. c., p. 284).

The question of the true nature of the species barrier has been studied by HIORTH, who used the method of attempting to transfer

certain important species characters of *amoena* to *Whitneyi*, first backcrossing the $F_1$ hybrid to *Whitneyi*, and then through successive generations crossing plants with the character studied to *Whitneyi*. Great difficulties have met the production of fertile *Whitneyi* plants having $F^b$ (HIORTH, 1946 b).

*Godetia amoena* × *G. Whitneyi* is very sterile. In the first backcross generation, $F'_2$, all $F^b$ plants were very sterile, while at least some $F^x$ plants were fertile. Also in $F'_3$ all $F^b$ plants had been very sterile. In the 3rd and following backcross generations certain distinctive $F^b$ types appeared, the properties of which will be discussed in the following pages. As a rule, $F^b$ plants also showed a pronounced pollen and seed sterility in the later generations, whereas $F^x$ and *f* plants were fertile. Selfing of the $F^b$ plants or crossings with these plants gave a low number of $F^b$ plants, $F^x$ or *f* plants appearing in a great excess. Thus the transfer of $F^b$ to *G. Whitneyi* has met with great difficulties, the $F^b$ plants in the crosses being more or less sterile and segregating only a low percentage of $F^b$. However, a fertile $F^b$ with a more normal segregation has at last appeared.

Many $F^b$ plants from the first and later backcross generations have been investigated cytologically, and my results are mentioned in the communication of HIORTH, but an illustrated description of the cytological observations is needed. Buds have been fixed (in Ås) of: (1) *amoena* × *Whitneyi* $F_1$ in 1941, (2) $F_1$ × *Whitneyi*, the first backcross generation, designated $F'_2$ in 1943, (3) the 3rd backcross generation $F'_4$ in 1941, (4) the 4th and 5th backcross generations $F'_5$ and $F'_6$, belonging to the same pedigree as $F'_4$, in 1942 and 1943. The genes involved in these crosses were $F^b$—$F^x$—*f* and $W'_1$—$w_1$. $W_1$ is one of three genes that cause a light violet instead of a white colour of the petals. $F^x$ and $W_1$ in *G. Whitneyi* show linkage with crossing-over values of more than 20 %. The *Whitneyi* chromosome having these genes has been designated 1.2 (HIORTH, 1946 c). $W_1$ from *amoena* was called $W'_1$.

*Godetia amoena* × *G. Whitneyi*, $F_1$. — Meiosis has been described several times. The hybrid is very sterile. HIORTH found a seed fertility of about 3 % and a pollen sterility of 94 %. The chromosome pairing is, however, good; the most common number of I's is 2. The hybrids investigated in 1931 showed III's, IV or a chain-of-five (HÅKANSSON, 1931); in *G. amoena* Sunol × *G. Whitneyi* Bremen no multivalents seemed to occur (HÅKANSSON, 1942). In a re-investigation of the latter hybrid, however, multivalents were detected. As is shown in Fig. 1 *a*,

at least 2 configurations-of-three may be formed. One is a heterochain with a rather small end-chromosome, the other is a homochain of about equal chromosomes. Sometimes there are 7 II; it seemed as though the small chromosome was paired with a chromosome from the latter configuration (Fig. 1 *b*). Often the small chromosome is univalent, the heterochain being instabile. The second configuration-of-three is more often observed. Rarely, a III with a triple chiasma is seen (Fig. 1 *c*), though it is uncertain whether this is a third configuration-of-three. An interstitial chiasma is sometimes observed in the chains (Fig. 1 *d*). Some p.m.c's with 5 II + chain-of-four were observed and very rarely a closed ring-of-four (Fig. 1 *e*). The configuration-of-four is more rare than the second configuration-of-three and, as Fig. 1 *f* shows, these two configurations may occur together. Another high catenation was chain-of-five. The structural differences between the standard genome of *Whitneyi* and *amoena* are also shown by the occurrence of unequal II's (2 or 3); very rarely is an inversion bridge seen. Fig. 1 *g* shows a somatic plate from a petal cell and gives an impression of the size relations of the chromosomes of the $F_1$ hybrid.

The occurrence of multivalents shows reciprocal translocations and duplications in the chromosomes. However, many p.m.c's have no multivalent, and »homologous» segments are thus often unpaired. Probably no p.m.c. was observed where all possible pairings were realized. HIORTH thinks that such a pairing would be ring-of-six + ring-of-four + two II's. A cytological demonstration does not seem to be possible, perhaps owing to this »asyndesis». In view of the strong structural differences and the absence of a large ring or chain that could bring about a regular assortment of the chromosomes, the high sterility of this hybrid is not surprising.

$F_1 \times G.$ *Whitneyi.* — The cross investigated had been of the type $(F^b\, W'_1 \times F^x\, w_1) \times f\, w_1$ and the offspring $1\, F^b\, F^x\, W'_1 : 22\, F^b\, W'_1 : 5\, F^b\, w_1 : 12\, F^x\, W'_1 : 36\, F^x\, w_1$. As HIORTH points out, crossing-over had been as high as in intraspecific crosses of *G. Whitneyi.* Buds from rather many plants had been fixed; the pollen fertility of these plants had been determined by HIORTH. Four $F^x$ plants were investigated. They were all cross-overs ($F^x\, W'_1$) and were pollen-fertile, having a pollen sterility of only 6 %. They formed 7 II and had a regular meiosis. No $F^x\, w_1$ had been fixed or had its pollen investigated. Some of them must have been sterile (p. 241). In mode of growth all $F^x$ plants were similar to *Whitneyi.*

All $F^b$ plants in the cross had a high pollen sterility, some had 70—

80 %, others had more than 90 % poor pollen. Only one of them had the mode of growth of *Whitneyi*, the rest were more similar to *amoena* in this respect. No $F^b$ had regularly 7 II and a regular meiosis; all investigated plants (19) showed the presence of *amoena* chromosomes.

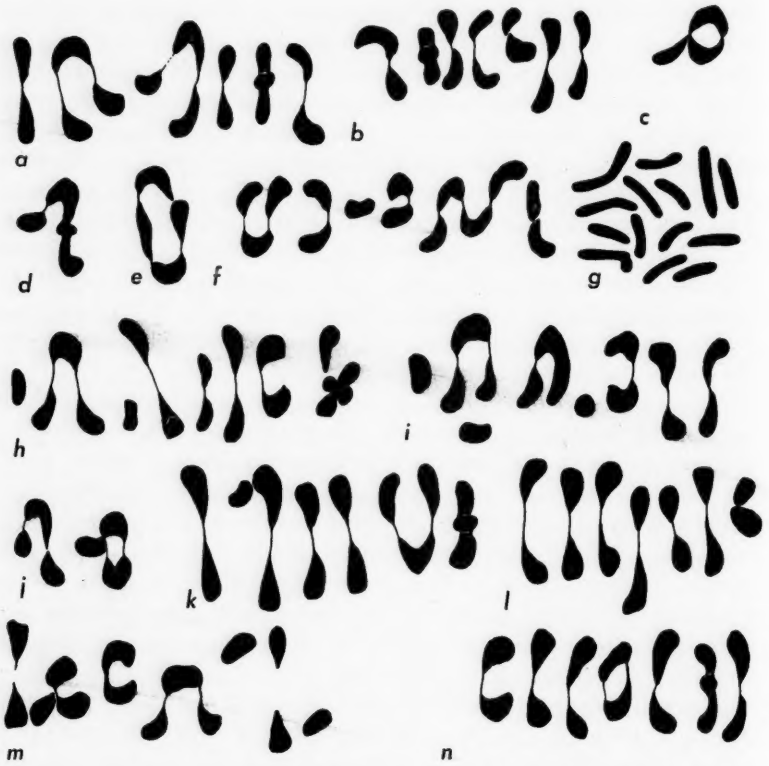


Fig. 1. *a—g. G. amoena* × *G. Whitneyi*. — *a*: 4 II + homo-III + hetero-III. — *b*: 7 II. — *c*: configuration with a triple chiasma. — *d*: heterochain showing an interstitial chiasma. — *e*: ring-of-four. — *f*: 1V + III + 2 II + 3 I. — *g*: somatic plate. — *h—n*. (*G. amoena* × *G. Whitneyi*) × *G. Whitneyi*, different $F^b$ plants. — *h*: 2678. 2 III + 4 II + 2 I. — *i*: 2696. 2 III + 3 II + 3 I. — *j*: 2660. heterochain + true III. — *k*: 2679. 4 II + hetero-III + homo-III. — *l*: 2679. 7 II. — *m*: 2680. 3 II + 2 I + 2 homo-III. — *n*: 2665. 7 II.

This was evident from the occurrence of multivalents or unequal II's or I's or an inversion bridge or a higher chromosome number than 14. CHITTENDEN (1928) has determined the somatic numbers in (*Whitneyi* ×

*amoena*) × *Whitneyi* and found 2*n* to be 16, 15 or 14. These numbers were here found in the $F^b$ plants. To describe meiosis in all these plants closely would be too burdensome; only a short summary will be given, and it must be remembered that the pairing was always variable.

One plant had 16 chromosomes; it was the $F^b F^x f$ plant (2678). As many as three III's could be observed, one of them being a heterochain — in Fig. 1 *h* the small chromosome is free from the chain. Sometimes a chain-of-four occurred. Four plants had 15 chromosomes. One of them (2663) formed only one configuration-of-three, a heterochain. However, this plant must have had more than one *amoena* chromosome because one could sometimes observe a chain-of-four. Two of the 15-chromosome plants rather often formed two configurations-of-three, a true III and a heterochain with a small end-chromosome, the latter seeming to be smaller than in $F_1$ (Fig. 1 *j* is from 2660). Plant 2696 also could form two chains, but the small chromosome seemed to be lacking here (Fig. 1 *i*); a chain-of-four rather often occurred. 2696 had much more often than $F_1$ a bridge and an acentric fragment. The reason is probably that here there is a new inversion that arose in $F_1$.

14 $F^b$ plants were »disomics». Most of them could form at least two configurations-of-three, a heterochain with a small end-chromosome and a homochain or true III with triple chiasma (Fig. 1 *k*). This pairing, which may be written 4 II + 2 III, was not so frequent. Other pairings were 5 II + I + III or 6 II + 2 I, univalents being of different size. The univalent could be small or large if only one chromosome was unpaired. A few plants of this type also had p.m.c's with 7 II, one II being heteromorphic (Fig. 1 *l*). Probably the small chromosome from the heterochain had paired with one of the chromosomes from the homochain; such a pairing could also occur in $F_1$. Some plants having two configurations-of-three were different; no clear case of a heterochain was observed, probably the small chromosome was not present. Fig. 1 *m* shows a pairing from such a plant, there is a true III and a chain of three chromosomes of about equal size.

Some disomics could show more than one unequal II. This is an indication that there are more *amoena* chromosomes than two. A higher number of *amoena* chromosomes must also be present in such disomics as could form a chain-of-four, an inversion bridge, or an usually high number of I's leading to a more or less frequent elimination at anaphase 1. The $F^b$ plant having a *Whitneyi*-like mode of growth had often 7 II's, two of them heteromorphic (Fig. 1 *n*), but could sometimes

form a homochain. This plant could also show an inversion bridge; the small chromosome was lacking.

All $F^b$ plants showed a chromosome number or a meiosis that indicated the presence of *amoena* chromosomes, and thus most probably the chromosome with $F^b$. The alleles $W_1$—$w_1$ were exchanged in $F_1$ but an exchange of $F$ alleles with loci in the same chromosome had not occurred. The high pollen sterility in $F^b$ plants is no doubt due to the *amoena* chromosomes present, these causing a structural hybridity that must result in a high haplontic sterility. As mentioned, there seemed to be different degrees of pollen sterility, 70—80 % and more than 90 %. Meiosis in the more sterile plants showed a greater structural hybridity and thus the presence of more *amoena* chromosomes than plants having the lower degree of pollen sterility, i. e. plants with a chain-of-four, with several heteromorphic II's or several I's were of the more sterile type.

In most cases the number of *amoena* chromosomes must be lower than in $F_1$ and meiosis is therefore less disturbed. Of more interest are such differences in the meiosis of backcross $F^b$ and $F_1$ as cannot be due to a different number of *amoena* chromosomes. Many $F^b$ plants could form two configurations-of-three, one of them a heterochain. Such a heterochain could also be observed in $F_1$, but more rarely. Thus, in disomic backcross $F^b$ p.m.c's with two configurations-of-three were more frequent than in $F_1$. The reason for this difference in pairing will be discussed in a following chapter. Metaphase 1 in $F_1$ often showed bivalents formed of chromosomes of different size and form. An interchange in such a heteromorphic II could lead to new chromosomes having a changed size or form. Such new chromosomes were, however, not detected in the backcross plants. A study of chromosomes in root tips would perhaps have shown that new chromosomes may be formed. This is not improbable, since some backcross $F^b$:s seemed to show a reduction in size of the small chromosome in the heterochain. This is, however, a point that will be discussed later. A more certain change is perhaps a new inversion in one of the plants.

All $F^b$ plants in the first backcross generation thus show sterility and the presence of *amoena* chromosomes. It is clear that the chromosome with $F^b$ causes sterility. Four $F^x$ plants were fertile and had a regular meiosis — 44 $F^x$ plants had, however, not been investigated. Now, HIORTH has made analogous crosses with the dominant *amoena* gene $C^{sp}$ that causes a violet dusting on the leaves. The first backcross generation showed that $C^{sp}$ segregates independent of $F^b$. $F^b$ $C^{sp}$ plants

always had, as expected, a high pollen sterility, but *f* $C^{sp}$ also was sterile. Out of 7 plants investigated, 2 had a pollen sterility of about 30 %, 5 of about 80 %. Meiosis in one $F^b$ $C^{sp}$ and one *f* $C^{sp}$ was studied. The former showed a rather small chromosome but was not investigated closely. As most common pairing *f* $C^{sp}$ had 5 II + I + chain-of-three. The chain was formed of chromosomes of about equal size (Fig. 2 *a*), a heterochain was never observed, the small chromosome was not present. Of very rare occurrence was a chain-of-four (Fig. 2 *b*). The chain seemed very similar to the homochain in $F_1$ and certain $F^b$:s in $F'_2$. Thus, it seems that the $C^{sp}$ chromosome from *amoena* also causes sterility when introduced into *Whitneyi* and is structurally different. Several $F^x$:s in the $F^b$ cross must have had this $C^{sp}$ chromosome and shown sterility.

($F_1 \times$ *G. Whitneyi*), $F'_4$. — The plants investigated belong to a different pedigree, the first backcross generation being sown in 1939. A number of $F^b$ plants in this and the following backcross generation had been pollinated with pollen from *G. Whitneyi* plants that were $F^x$ or *f*. In this way $F'_4$ (sown in 1941) consisting of six families having 15 $F^b$ : 141 non-$F^b$ had arisen. All $F^b$ plants in this as in the preceding generations were sterile. They had a long bent stem like *G. amoena*. One $F^b$ (3054/795) had, however, a mode of growth more similar to that of *G. Whitneyi* and had a better fertility than other $F^b$ plants in $F'_4$. Six plants were investigated.

Plant 3054 had 15 chromosomes and the pairing 6 II + heterochain (Fig. 2 *c*). The heterochain will be described later; it is composed of chromosomes that all are different. The small chromosome was sometimes unpaired, and the univalent formed may be divided or eliminated at anaphase 1 or 2. The absence of more I's and the fact that none of the II's showed any heteromorphy indicate that the six II's were formed by *Whitneyi* chromosomes. As will be shown below, the heterochain very probably has a *Whitneyi* chromosome in the centre, while the end-chromosomes are *amoena* chromosomes.

Four plants (3052, 3053, 3055, 3056) had a similar cytology. They had 14 chromosomes and could form two configurations-of-three. One of these configurations was the heterochain that was also formed by the trisomic plant. The small end-chromosome is smaller than the other chromosomes; this is also evident from somatic plates (Fig. 2 *i*). The second configuration-of-three is formed by two equal and one slightly smaller chromosome (Fig. 2 *d*). This configuration was very instabile; in most p.m.c's one of its chromosomes was univalent and hence this

configuration is rather difficult to study. Probably it is formed of one *amoena* and two *Whitneyi* chromosomes, but it seems that a triple chiasma may rarely be formed. The small chromosome from the heterochain is also rather often univalent, and the pairing 6 II + 2 I is frequent, the I's being of a very unequal size (Fig. 2 *e*). Only

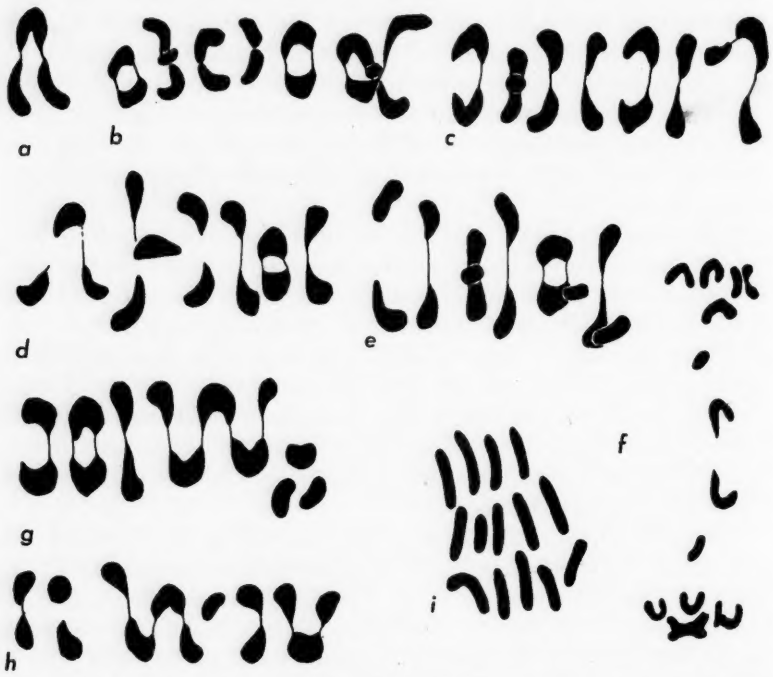


Fig. 2. *a—b.* (*G. amoena* × *G. Whitneyi*) × *G. Whitneyi*, *f* $C^{sp}$ plant. — *a*: homo-III. — *b*: 5 II + IV. — *c—i*: (*G. amoena* × *G. Whitneyi*) × *G. Whitneyi*, $F^b$ plants from $F_3$'s. — *c*: 6 II + heterochain. — *d*: 4 II + III + heterochain. — *e*: 6 II + large I + small I. — *f*: division of I's at first anaphase. — *i*: somatic plate. — *g—h*: plant 3058. — *g*: V + 3 II + 3 I. — *h*: V + III + 3 II.

occasionally do more than 2 I's occur. Fig. 2 *f* shows the division of the two I's at anaphase 1.

One $F^b$ plant (3058) was a disomic having a different pairing. In many p.m.c's a chain-of-five could be observed with three large chromosomes in the centre and smaller end-chromosomes (Fig. 2 *g*). One end-chromosome in this chain was particularily small. As is shown in Fig. 2 *h*, this chain and a chain-of-three may occur in the same p.m.c.

The longer chain could be divided into a chain-of-four + I or a chain-of-three + II. This plant frequently had more than two I's and anaphase 1 was often rather disturbed.

The more sterile $F^b$ plants were disomics and had more *amoena* chromosomes than 3054, this being shown by the formation of a second configuration-of-three. The great structural hybridity that thus is evidenced by the pairings observed explains this great sterility of the disomics. A greater number of *amoena* chromosomes also explains the *amoena*-like or intermediary mode of growth of the disomics, while 3054 was more similar to *Whitneyi*. It seems probable that the sterile $F^b$ plants that have not been investigated were also disomics, most of them perhaps having the two configurations-of-three. The disomic 3058 with the chain-of-five came from a very sterile mother plant. As the offspring in this case was from an unisolated branch, pollen may have come from a *Whitneyi* with a chromosome ring. However, the assumption of the occurrence of a new translocation is needed to explain this unexpected pairing (cf. p. 253).

All II's in the disomic $F^b$ were no doubt formed by *Whitneyi* chromosomes, this in contradistinction to the disomics with two configurations-of-three in $F'_2$ that could show evidence of more *amoena* chromosomes. In no case was a heteromorphic II formed by chromosomes belonging to different chains observed, and the small chromosome in the heterochain here seemed to be smaller than the smallest chromosome in the $F_1$ hybrid.

($F_1 \times G.$ *Whitneyi*), $F'_5$ and $F'_6$. — In $F'_4$ there seemed to be two different types of $F^b$. One plant (3054) represented what HIORTH has called the heterotrisomic $F^b$ type having 6 II + heterochain, the others were of what HIORTH called the disomic sterile $F^b$ type having the heterochain and a second configuration-of-three. These types were also found in later backcross generations, but while in $F'_4$ most $F^b$ plants were of the disomic sterile type, only one of the $F^b$:s from later generations that had been fixed belonged to this type. The heterotrisomics are more fertile and are similar to *G. Whitneyi* in mode of growth. HIORTH found in 54 plants of the latter type 25—81 % poor pollen with a mean of 47 % (all these plants had not been investigated cytologically). Plants of the sterile type had more than 80 % poor pollen and always had a much lower seed fertility than the heterotrisomics. Thus, six sterile $F'_4$ plants after backcrossing gave 21 $F^b$ : 92 non-$F^b$, while the one heterotrisomic gave 20 $F^b$ : 70 non-$F^b$.

From the offspring last mentioned (the family 882—1942) that had

3054 as mother three $F^b$ plants were studied. In the next backcross generation $F'_6$ the segregation had been in all 26 $F^b F^x$ : 376 $F^x F^x$. A few $F^b$ individuals from this generation (808—1943) were also investigated. All plants were heterotrisomics, and meiosis could be studied more thoroughly than in 3054. The small end-chromosome in the

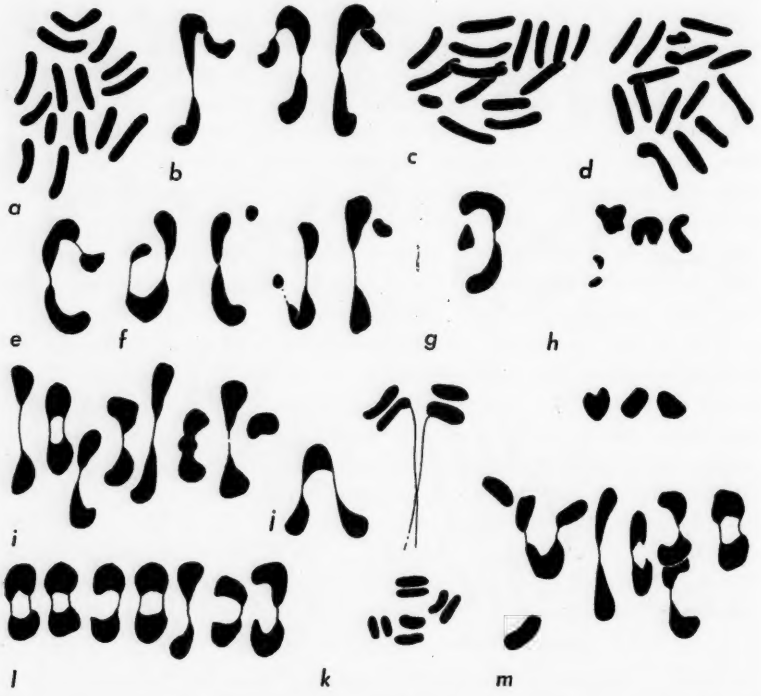


Fig. 3. (*G. amoena* × *G. Whitneyi*) × *G. Whitneyi*. $F'_5$ and $F'_6$. — *a*: heterotrisomic $F^b$, somatic plate. — *b*: heterotrisomic $F^b_1$, heterochains. — *c*: $F^x$ plant 1138/886, somatic plate. — *d*: $F^b$ plant 1131/886, somatic plate. — *e*: 1138/886, heterochain. — *f—g*: $F^b$ from 846—1943, heterochains. — *h*: 845—1942, elimination of fragment chromosome at first anaphase. — *i—k*: the $F^b$ plant 1171/889. — *i*: III + 6 II. — *j*: the configuration-of-three. — *k*: bridge formation at first anaphase. — *l*: fertile $F^b$, 7 II. — *m*: the $F^b$ plant 2669/817—1943, III + 5 II + 2 I.

heterochain measured 7 mm. at a magnification of × 5000, the larger end-chromosome 12 mm. The former had a submedian centromere, the latter a subterminal. The chromosome in the centre was heterobrachial, the short arm formed chiasma with the small chromosome. The centro-

meres of the end-chromosomes are orientated towards one pole, the chromosome in the centre towards the opposite pole. This must cause a rather constant distribution of the chain at anaphase 1, the end-chromosomes going to the same pole. Exceptions from this distribution may occur. One cause of such exceptions is a more neutral orientation of the small chromosome, its centromere not being directed towards any definite pole (cf. Fig. 3 *g*), another cause is univalence of the small chromosome. In one slide as many as $^1/_3$ of the p.m.c's had a small I, but other slides showed a lower frequency, and an elimination of the small chromosome occurred in less than 20 % of the p.m.c's.

The appearance of the chain was somewhat variable. The small chromosome could be connected by a thread but perhaps more often the connection was closer (Fig. 3 *b*). The third mode of connection, through an interstitial chiasma, was never observed, nor was such a chiasma formed by the larger end-chromosome. The II's are as a rule open, only 0—2 ring-II's were observed in the p.m.c. A somatic plate is shown in Fig. 3 *a*. It is very probable that all $F^b$:s in these families were heterotrisomics.

An interesting $F'_5$ family was 886—1942 from a cross $F^bF^x \times ff$. The segregation was 5 $F^b f$ : 14 $F^x f$. The mother plant, 3051/795—1941, had unfortunately not been investigated. HIORTH mentions that it belongs to the sterile, disomic type. All $F^b$ and 5 $F^x$ plants were investigated. The $F^b$ plants were heterotrisomics, and the small chromosome had a reduced size (Fig. 3 *d*). While, as a rule, it has more than half the length of the larger end-chromosome, it is here less than half of this chromosome, at a magnification of 5000 measuring 4,5—5 mm. at first metaphase. The orientation of the chromosomes of the heterochain is the usual, at anaphase 1 the end-chromosomes pass to the same pole. Here, more often the small chromosome is at a distance from and connected by a thread to the chromosome in the centre.

Four $F^x$ plants had, as expected, 7 II and had no doubt 14 *Whitneyi* chromosomes. One $F^x$ (plant 1138) was, however, heterotrisomic; it had the small chromosome (Fig. 3 *c*). The heterochain was somewhat different from the chain of the $F^b$ sisters. It was the larger end-chromosome that was different, this having the same size and form as the chromosome in the centre (Fig. 3 *e*). The chain thus has two *Whitneyi* chromosomes, and 1138 as normal $F^x$ plants 14 such chromosomes. The number of ring II's were rather high, 3—4 in most p.m.c's. Plant 1138 had a pollen sterility of 27 %, while four normal $F^x$ sisters had only 3—6 %. After selfing, 1138 has given 152 $F^x W_1$ : 21 $F^x w_1$ : 18 $f W_1$ :

29 *f* $w_1$. This segregation is quite normal and shows that the small chromosome has no influence on the segregation (HIORTH, l. c.). The cause of the pollen sterility is unknown. The small chromosome was often univalent.

The $F^b$ plant 1131/886 had in a cross $F^b f \times ff$ unexpectedly given the segregation 24 $F^b f$ : 35 *f f* (= the family 846—1943). Selfing of 1131 also gave an unexpectedly high number of $F^b$ plants. Of 846—1943 one *f* and seven $F^b$ plants were investigated. The *f* had 7 II, all $F^b$:s were heterotrisomics with a very small chromosome (Fig. 3 *f*), similar to that in the mother plant. A division of the fragment chromosome is shown in Fig. 3 *h*. The $F^b$ plants in 846 seemed to be very sterile, five plants pollinated with *f* pollen giving offspring of only 103 plants, 6 $F^b f$ : 97 *ff*. This is again unexpected, the number of $F^b$ now being low, perhaps owing to the sterility (HIORTH). The unusual segregation of 1131 could not be due to the reduced size of the small chromosome because sister plants have given the ordinary heterotrisomic segregation.

The small chromosome of the heterochain may also be larger than in 882—1942; this was possibly the case in the only $F^b$ plant in 814—1943. The mother plant had not been investigated but was of the sterile type (HIORTH).

A cross of the type $F^b F^x \times ff$ had given 1 $F^b F^x f$ : 1 $F^b f$ : 19 $F^x f$ (the family 889—1942). The mother plant was 3056 from $F'_4$, a disomic having two configurations-of-three. Plant $F^b f$ was a heterotrisomic with the usual heterochain. Plant $F^b F^x f$ (1171/889) also had 6 II and a configuration-of-three, but it was not the heterochain, being formed of two chromosomes of equal size and one that was slightly smaller (Fig. 3 *i* and *j*). A triple chiasma was sometimes observed. The pairing could also be 7 II + I or 6 II + 3 I. Anaphase 1 was very disturbed, there being frequent laggings and eliminations. Bridges were observed, but no acentric fragments. Fig. 3 *k* shows a bridge uniting two chromosomes from the III going to the same pole. It is clear that 1171 lacks the small chromosome, and the constitution $F^b F^x f$ shows that a non-disjunction had probably occurred in the mother plant. Thus, the new configuration was probably composed of two *Whitneyi* chromosomes and the larger end-chromosome from the heterochain, because in the mother plant the chromosome in the centre and the larger end-chromosome had gone to the same pole. 1171 is the homotrisomic $F^b$ type of HIORTH.

889—1942 showed that a disomic $F^b$ had produced a heterotrisomic $F^b$. In a similar cross with a cytologically investigated disomic $F^b$ from

$F'_4$ only one $F^b$ was examined and this plant had 4 II + 2 configurations-of-three as the mother plant (888—1942).

In $F'_5$ appeared the first $F^b$ plant that was fully fertile: the fertile $F^b$ type. This plant had a pollen sterility of only 2 %, after selfing it gave 67 $F^b F^b$ : 213 $F^b F^x$ : 115 $F^x F^x$, that is 71 % $F^b$, 17 % $F^b F^b$, while the heterotrisomic segregates 12,2 % $F^b$ and 0,21 % $F^b F^b$. The fertile plant (1107/883—1942) was not investigated, however, two $F^b F^b$ and two $F^x F^x$ individuals from the progeny showed 7 II (Fig. 3 *l*) and a regular meiosis; an $F^b$ plant from Bremen × 1107 also had 7 II. There was no trace of heteromorphy in any II, and the fertile $F^b$ no doubt had only *Whitneyi* chromosomes.

HIORTH has obtained the fertile $F^b$ type 12 different times; it has appeared in the offspring from heterotrisomic $F^b$, disomic sterile $F^b$ or the homotrisomic 1171. Plants belonging to eight of these lines have been investigated, they all had 7 II, in certain plants a weak asyndesis sometimes causing I's was manifested. The segregation of the fertile type is normal. In several cases HIORTH, however, found too few $F^b F^b$ plants. Crossing-over in $F^b$—$W_1$ was normal (26 %). Some plants had a certain amount of pollen sterility (in one case 43 %).

Finally, the family 817—1943, the cross *ff* × 1106/883, may be mentioned. The male parent 1106 had not been investigated; it was a sterile $F^b$. The $F^b$ plants in 817 showed a very different sterility. One plant had a pollen sterility of 7 % and had 7 II. One plant having a pollen sterility of 73 % was a trisomic with a heterochain. Elimination was rather frequent at anaphase 1, and bridges were seen. A third plant (2269/817) with a sterility of 38 % was a trisomic that formed a chain of chromosomes of rather equal size. One of these chromosomes was rather often univalent and could be eliminated at anaphase 1. In a letter HIORTH has communicated the results of crossings with this plant. Selfing of 2669 has given 13 $F^b W_1$ : 2 $F^b w_1$ : 10 $f W_1$ : 26 $f w_1$, and $f w_1$ × 2669/817 gave 56 $F^b W_1$ : 5 $F^b w_1$ : 19 $f W_1$ : 176 $f w_1$. Several $F^b$ plants in the offspring showed a similarity to *G. amoena*. HIORTH finds these results indicate that 2669 is a $F^b$ of the fertile type. The configuration-of-three was, as a rule, a chain having a rather large chromosome with a submedian centromere in the centre, the end-chromosomes being slightly smaller. These latter are perhaps *amoena* chromosomes (Fig. 3 *k*). The same configuration must have been present in 1106/883.

*Conclusions.* — With the production of a fertile $F^b$ type the problem of transferring $F^b$ to *G. Whitneyi* has been solved. To begin with, how-

ever, the interesting heterotrisomic $F^b$ type that first appeared in $F'_4$ will be discussed. The heterotrisomic cannot have more than two *amoena* chromosomes, the end-chromosomes of the heterochain. As a rule, it forms two kinds of fertile gones, $F^x$ or *Whitneyi* gones having 7 chromosomes including the chromosome in the centre of the heterochain, and $F^b$ gones having 8 chromosomes including the end-chromosomes of the chain. Elimination of the small chromosome must rather often cause the formation of gones having 7 chromosomes including the large end-chromosomes but lacking the small. The results show that such gones must be sterile. Also a gone with the small chromosome and having only 7 chromosomes is sterile, for no plant with 13 + the small chromosome has been found. Thus the two end-chromosomes in a way complement one another to substitute the *Whitneyi* chromosome of the heterochain and give a fertile gone. As most sterile $F^b$ plants had the small chromosome, one might be inclined to think that the gene $F^b$ is in the small chromosome. However, this does not seem to be the case, $F^x$ having the small chromosome has been found, and also $F^b$ (sterile) lacking the small chromosome. A view more in harmony with the observations is that $F^b$ has its locus in the larger end-chromosome — the presence of the small chromosome in most $F^b$ plants is a result of the distribution of the chromosomes of the heterochain and of the fact that it supplements the $F^b$ chromosome to give a fertile gamete. HIORTH designated the small chromosome 2.3', the larger end-chromosome 1.6', the *Whitneyi* chromosome being 1.2 (Fig. 4). The heterochain, thus, is 6'.1—1.2—2.3', the ends marked ' are according to HIORTH not able to form a chiasma with corresponding *Whitneyi* segments. The sterility of $F^b$ gones with 7 chromosomes is due to the absence of segment 2. The chromosomes of *G. Whitneyi* have been numbered 1.2, 3.4, 5.6, and so on. One could never observe the heterochain in connection with any of the 6 *Whitneyi*-II's. This must mean that the free ends of the two *amoena* chromosomes cannot form a chiasma with »homologous» *Whitneyi* ends 3 and 6.

The assumption that the *F* alleles have not loci in the chain but in a bivalent, and that the $F^b$ chromosome of this bivalent must be supplemented by the small chromosome, is in conflict with many facts. Then the III of $F^b F^x f$ (plant 1171/889) would be composed of three identical chromosomes, which, however, is not the case.

The small chromosome is more or less frequently unpaired. In view of its small size and of the fact that it comes from a foreign genome this is not surprising. In $F_1$ the corresponding chromosome

was still more often univalent, or it could form a II with a chromosome from a different configuration-of-three; a heterochain was rare in $F_1$. The larger *amoena* chromosome was never unpaired in the heterotrisomic. But also in $F_1$ it was probably rather rarely univalent: in $F_1$ a regular crossing-over of the genes $W'_1$ and $w_1$ linked to $F$ alleles is as high as in intraspecific crosses with races of *Whitneyi*. This indicates a regular pairing between these chromosomes in the $F_1$ hybrid; $W'_1$—$w_1$ must have loci near the ends of the chromosome arms that form the chiasma, that is, near the end of the longer arms 1. An interchange must here be frequent, but it would by no means be surprising if the pairing of these arms was still more frequent in later generations, these interchanges having made the long arm of the $F^b$ chromosome more similar to the homologous *Whitneyi* arm. Frequent interchanges do not, however, occur in the part of the chromosomes containing loci of the $F$ alleles. Exchanges between the larger end-chromosome and the *Whitneyi* chromosome of the chain perhaps also explain the absence of an interstitial chiasma in later generations.

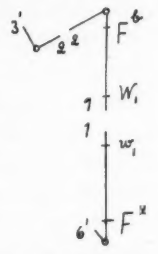

Fig. 4. Diagram of the heterochain.

The pollen sterility of heterotrisomic $F^b$ must be caused by the elimination of the small chromosome. However, many trisomics had a much higher pollen sterility than one would expect after the elimination observed. HIORTH assumes that secondary causes often increase this primary elimination sterility. The rather low number of $F^b$ plants in the offspring is explained by the fact that $F^b$ gones have $n + 1$ chromosomes, two of them coming from a foreign species. When used as male parent, the heterotrisomic usually gives only slightly more than 1 % $F^b$; the transmission of $F^b$ through the egg-cells is better, selfing giving 12,2 %.

At least in two cases an unusual distribution of chain chromosomes has resulted in aberrant plants, once an $F^x$ having 14 *Whitneyi* chromosomes and the small chromosome, and once an $F^b$ having 14 *Whitneyi* chromosomes and the $F^b$ chromosome (plant 1171/889; p. 246). In the former case the small chromosome had passed to the same pole as the chromosome in the centre of the chain, in the latter case the larger end-chromosome had followed the chromosome in the centre. 1171/889 was $F^b\,F^x\,f$, and it seemed unable to transmit the $F^b$ chromosome through the pollen. In crosses where 1171 is the male parent it has given $0\,F^b\,F^x : 3\,F^b : 222\,F^x : 177\,f$. One $F^b$ was investigated; it

had 7 II and thus belonged to the fertile type lacking the $F^b$ chromosome 1.6'. Probably the two $F^b$:s not investigated also had 7 II. Selfing 1171 has given 8 $F^b$ : 110 non-$F^b$; an $F^b$ investigated had 7 II. It seems, however, probable that egg-cells having 7 *Whitneyi*- + the $F^b$-chromosome may function, though more rarely than egg-cells with 6 *Whitneyi*- + the $F^b$ + the small chromosome in heterotrisomics.

The fertile $F^b$ plants had 7 II. No doubt the fertile type has only *Whitneyi* chromosomes, crossing-over in the heterochain having transferred the gene $F^b$ to the chromosome 1.2. In all plants with a chain having the $F^b$ chromosome 1.6' crossing-over could occur, and fertile $F^b$ has been produced by disomic sterile $F^b$, heterotrisomic $F^b$ and the »homotrisomic» 1171, as one could expect. Relatively often it has appeared in crosses where heterotrisomic $F^b$ was the male parent. HIORTH attributes this to the fact that the cross-over pollen grains must be more vital than the usual $F^b$ grains with 8 chromosomes. However, crossing-over must be very rare. $W_1$ of the same linkage group had, as mentioned, more than 20 % crossing-over and this difference must be due to a different position of the genes, $F$ alleles being nearer the centromere, $W_1$ near the end of the long arm of the chromosomes (Fig. 4). It is improbable that $F^b$ has its locus in the short arm, no chiasma being observed there and these arms being of different size in 1.2 and 1.6'. HIORTH has shown that in the fertile type $F^b$ must be allelic to or show absolute linkage with $F^x$ of *Whitneyi*, as crosses $ff \times F^b F^x$ have given 356 $F^b f$ : 447 $F^x f$. In most crosses with the fertile type too few $F^b F^b$ plants appear; the transmission of $F^b$ is better through the pollen than through the egg-cells.

On p. 247 reference is made to plant 2669/817, which segregates like the fertile type but has *amoena* chromosomes. There was probably a cross-over chromosome in the pedigree, as also seems to be evident from the repeated production of fertile $F^b$ plants. This chromosome was probably in the $F'_4$ plant 3048—1941 (not investigated), 3048 $\times$ $F^x$ had produced 3 $F^b$ plants, one fertile (7 II), two very sterile. One of the sterile plants was mother of the heterotrisomic in 814—1943. The second sterile plant (1106) produced several fertile $F^b$:s. In the offspring after selfing 1106 two $F^b$:s with 7 II were found, and in $f \times$ 1106 one heterotrisomic $F^b$ and two having the gene $F^b$ in a bivalent. These facts are perhaps explicable on the assumption that 3048—1941 had a heterochain with a cross-over chromosome in the centre and a second configuration, viz. the one shown in 2669/817.

There is a disomic $F^b$ type which is very sterile. Heterotrisomics

could also show a rather high pollen sterility but always had a much lower seed sterility. The disomics had the heterochain, but a second configuration-of-three showed the presence of more *amoena* chromosomes. This configuration is probably composed of 2 *Whitneyi* chromosomes and 1 *amoena* chromosome. It has been shown that in the offspring of $F^b$ disomics of this cytological type there may be $F^b$ plants with the same pairing, heterotrisomics, »homotrisomics» and fertile $F^b$:s. If the end-chromosomes of the heterochain and the two *Whitneyi* chromosomes in the second configuration-of-three go to the same pole, gones are formed giving heterotrisomic $F^b$ in the backcross. If it is the *amoena* chromosome of the second configuration that follows the end-chromosomes of the heterochain, then a plant like the mother is formed. The *amoena* chromosomes are no doubt the cause that $F^b$ gones function much more rarely than $F^x$ gones having 7 *Whitneyi* chromosomes. In later generations disomic sterile $F^b$ plants seem to be rare, and such plants are of course never formed by heterotrisomic $F^b$.

HIORTH has preliminarily numbered the chromosome ends in the second configuration as 3.4—4.5—5.6, the chromosome 4.5 being from *amoena*. This configuration could not be studied so well as the heterochain; it is more instabile and may show a triple chiasma, indicating a more complicate structure than these numbers show. A number of the plants that have been used in crosses and that HIORTH considers to be of the disomic, sterile type have not been investigated. All disomic sterile $F^b$ plants do not show this pairing; some perhaps had more than three *amoena* chromosomes. The second configuration is different from the homochain in $F_1$ and $f\ C^{sp}$.

The small chromosome may have a different size. It cannot be doubted that in $F'_4$—$F'_6$ it is smaller in most families than in $F_1$. In a pedigree coming from plant 3051/795—1941 it was particularly small. Here the mother plant must have a chromosome of diminished size. The cause of this variation in size of the small chromosome is perhaps an exchange with the chromosome in the centre, this exchange being unequal. Exchanges between these two chromosomes may also explain the fact that a chain with a small chromosome that is rather rarely formed in $F_1$ is more often observed in certain plants of $F'_2$ and still more often in later backcross generations. A second cause of the rareness of heterochains in $F_1$ and certain $F'_2$ plants is that the small chromosome sometimes forms a bivalent with a larger chromosome from a homochain or III. Such a behaviour of the small chromosome was not observed in $F'_4$—$F'_6$, the second configuration-of-three of these di-

somics is, as it seems, not identical with the homochain or III so often observed in $F_1$ or $F'_2$ and in *f* $C^{sp}$. In $F_1$ and $F'_2$ one would sometimes expect a chain-of-six; such a configuration has, however, not been observed.

Exchanges between *amoena* and *Whitneyi* chromosomes in the heterochain might thus give an explanation not only of the better pairing but also of the size reduction of the small chromosome. But unequal exchanges should also give rise to a »small» chromosome increased in size. Whether such an increase has actually occurred is not clear.

Two *amoena* chromosomes have caused pollen sterility in backcrosses to *Whitneyi*, the $F^b$ chromosome and the $C^{sp}$ chromosome. The former is a member of a heterochain and thus structurally different from homologous *Whitneyi* chromosomes, the latter is also structurally different, being very probably a member of a chain-of-three or -four.

DOBZHANSKY speaks of two different kinds of hybrid sterility, genic and chromosomal. At first the sterility of the $F^b$ plants in the crosses seemed to be a genic sterility; it is, however, a case of chromosomal sterility, crossing-over of the gene to a *Whitneyi* chromosome having removed the cause of sterility.

Probably still more *amoena* chromosomes produce sterility when introduced into the *Whitneyi* genome. Other *amoena* chromosomes may be structurally similar to and regularly pair with the corresponding *Whitneyi* chromosome. This is probable also from the fact that the number of I's in $F_1$ is always low, and some II's seem to be strictly homomorphic. Such chromosomes may easily replace homologous *Whitneyi* chromosomes. But clearly the important morphological species differences in *amoena—Whitneyi* have no loci in such easily interchanged chromosomes. It is true that many structural differences may occur in intraspecific races or hybrids in *G. Whitneyi*, but the rather constant ring formation and a regular assortment of ring chromosomes here results in fertile gones. In the interspecific crosses larger rings are not formed, certain »homologous» segments often or always being unpaired and the result being a more independent distribution of the structurally different chromosomes leading to haplontic sterility. It has been mentioned that HIORTH considers the pairing 2 II + ring-of-four + ring-of-six as that which would be shown by *amoena* × *Whitneyi* if all homologous ends were united at metaphase 1. The ring-of-four should contain the $C^{sp}$ chromosome, the ring-of-six the chromosomes of the two configurations-of-three in disomic sterile $F^b$.

In two cases chromosome changes not involving the small chromosome had manifested themselves. One was a new inversion in $F'_2$ and the second was a reciprocal translocation involving the 6' end in $F'_4$. Plant 3058, mentioned on p. 242, had a chain-of-five with 2 small end-chromosomes. One end-chromosome was probably 2.3' and therefore the $F^b$ chromosome should be the chromosome in the centre of the chain. This chromosome is (a) larger than the normal $F^b$ chromosome 1.6' and has a submedian centromere, (b) forms a chiasma in both its arms. These facts speak in favour of the view that the chromosome 1.6' has made a reciprocal exchange involving the end 6' that now should belong to the second end-chromosome.

## SOME NEW CASES OF SPONTANEOUS CHROMOSOME CHANGES IN GODETIA.

That the chromosomes of *Godetia* must be very liable to structural changes is already indicated by the common occurrence of rings or chains at metaphase 1. More rare than reciprocal translocations are inversions. Fragmentations or deletions where a chromosome shows a reduced size while a corresponding increase in size in other chromosomes could not be observed, have been found after X-raying. HIORTH produced aberrant plants of *G. Whitneyi* by pollination with X-rayed pollen. Three aberrants had a diminished chromosome (HÅKANSSON, 1940). One was a trisomic with a very small chromosome that sometimes formed a hetero-III with two chromosomes of normal size. Two were disomics that often formed a hetero-II; in one of them the fragmented chromosome was very small. A spontaneous chromosome fragmentation has been found in the allotrisomic *Rn* (HÅKANSSON, 1941), and some new cases of this type will be described here because they were accompanied by certain genetical peculiarities.

Backcrossings of the type (*Godetia deflexa* × *G. Whitneyi*) × *G. Whitneyi* have produced several different allotrisomic types having 14 *Whitneyi* chromosomes and 1 *deflexa* chromosome (HIORTH, 1946 a). At first metaphase they have the pairing 7 II + I (HÅKANSSON, 1941). They are similar to *G. Whitneyi* but show certain morphological characters from *deflexa*. Each of them must have a different *deflexa* chromosome and is called after the most striking *deflexa* character shown.

The allotrisomics described by HIORTH are: (1) *Kl* (= kleinblütig) has small flowers, a very high pollen sterility (98 %), in *Kl* ♀ × normal ♂ there is an excess of *Kl* plants (62 instead of 50 %), (2) *Rn* (= rotnervig), the middle rib of the leaves

has a red colour; *Rn* ♀ × normal ♂ gives too few *Rn* plants (11,8 %); in normal ♀ × *Rn* ♂ only 3,5 % *Rn* appear. X-raying the pollen of *Rn* has produced a translocation called $Rn^t$, a segment of the *deflexa* chromosome had been translocated to a *Whitneyi* chromosome. $Rn^t$ has only 14 chromosomes and forms 7 II, one of them a hetero-II (HÅKANSSON, 1943). In $Rn^t$ ♀ × normal ♂ a much higher number of plants appear with red ribs on the leaves (39,9 %), in normal ♀ × $Rn^t$ ♂ 10,9 %. Homozygous *Rn* is unknown. $Rn^t\ Rn^t$ is, however, vital. It has no hetero-II (HÅKANSSON, 1944) and is a rather weak type, having smaller flowers and leaves than and being only about half the height of heterozygous $Rn^t$. (3) *Pu* (= punktiert) has violet points on the petals. In *Pu* ♀ × normal ♂ only 6,9 % are *Pu*, and in crosses of the type normal ♀ × *Pu* ♂ there is only 0,13 % *Pu*. Spare pollination, which in the cross normal ♀ × *Rn* ♂ considerably increases the frequency of *Rn*, is of no influence here. HIORTH suggests as an explanation that the growth of the pollen tube is entirely dependent on the tube nucleus, that *Pu* is lethal to the tube, and that the few *Pu* plants formed are the result of an elimination of the *Pu* chromosome at the first pollen mitosis, with the result that a *Pu* chromosome is present in the generative nucleus but not in the tube nucleus.

A plant with a diminished chromosome was detected in $F_1$ from a cross between *Kl* and a recessive *Whitneyi* strain *gg* . *gg* plants have a green-light red stem, while *G* causes a red stem colour. In *Kl GG* × *gg* one of the disomic $F_1$ plants had, however, a green stem showing the recessive character (HIORTH, 1946 a). This plant was a variant having reduced size and buds, more hairy than usual. The cytological investigation showed that this plant had 14 chromosomes, one of them being very small. In Fig. 5 *e* is a plate from a young petal; the small chromosome is about $^1/_4$ of a normal chromosome. Figs. 5 *a*—*c* show the chromosome pairing at metaphase 1. In *b* the fragment chromosome is paired with a normal chromosome. However, in most p.m.c's the fragment chromosome is unpaired; in Fig. 5 *a* there is 6 II + I + fr., and in *c* chain-of-three + 5 II + fr. Fig. 5 *d* shows late anaphase 1; the fragment chromosome is belated. It may be added that in a few loculi the fr. chromosome seemed to be absent, indicating that it had been eliminated at mitosis.

It is clear that this fragment chromosome has been formed by a maternal *G* chromosome which has lost most of its substance, including the gene *G*.

A second case of a spontaneous chromosome fragmentation is in $Rn\ ^tRn^t$. HIORTH has crossed this form and the standard race Bremen. In $Rn^t\ Rn^t$ ♀ × Bremen ♂ all $F_1$ plants (1000) were $Rn^t$, as expected. Three of them were investigated and the heteromorphic II that characterizes heterozygous $Rn^t$ could be observed in all. Reciprocal crosses, Bremen ♀ × $Rn^t\ Rn^t$ ♂, gave progenies of 2003 plants; however, no fewer than 54 had a green rib on the leaves, thus unexpectedly showing

the recessive character. I have investigated ten of these aberrant plants. Two of them were haploids (the haploidy was suspected by HIORTH); they have arisen by parthenogenesis. Eight of them were disomics with 7 normal II's; no hetero-II could be observed. It is clear that the translocated *deflexa* segment is not present in the aberrant $F_1$ plants with green ribs on the leaves. In the homozygote $Rn^t\ Rn^t$ the *Whitneyi* chromosome carrying the *deflexa* segment is liable to changes leading to the loss of this segment.

Fig. 5. *a—e.* Allotrisomic *Kl GG* × *gg* $F_1$, *gy* plant. — *a*: 6 II + I + fr. — *b*: 7 II. — *c*: III + 5 II + fr. — *d*: elimination of fr. chromosome at first anaphase. — *e*: somatic plate. — *f—h. G. Whitneyi*, trisomic $C^{pa}$, plant with $C^{pa}$ fragment chromosome. — *f*: somatic plate. — *g*: 6 II + 2 I + fr. — *h*: hetero-III.

Two of the green-ribbed plants were semisterile. HIORTH suggests that this sterility can be explained by the assumption that a minor part of the *Whitneyi* chromosome was lost at the same time as the *deflexa* segment. In several plants a number of the p.m.c's had 6 II + 2 I. This was also found in 15 % of the p.m.c's of $Rn^t\ Rn^t$. The cause of this asyndesis is not clear. A strange effect of the rather frequent loss of the *deflexa* segment is that homozygous $Rn^t$ is not quite constant. In the progeny of $Rn^t\ Rn^t$ HIORTH detected some $Rn^t$ heterozygotes, that is, plants distinctly larger than the homozygotes. Selfing of these larger plants resulted in an offspring segregating in plants with red and with green ribs, and thus the forming of $Rn^t$ heterozygotes from $Rn^t$ homozygotes is proved.

It is interesting that the foreign segment seems to be rather loosely attached to the *Whitneyi* chromosome. Probably the loss of the segment occurs at meiosis in the p.m.c., but this has not actually been observed in the slides. There is so far no evidence that the segment also may be lost in the e.m.c. HIORTH, however, stresses the fact that pollen tubes with $Rn^t$ do not function so well as normal tubes, backcrosses with the heterozygote as male parent giving only 10,9 % $Rn^t$ instead of, as expected, 50 %. Loss of the segment may thus give rise to pollen grains functioning far better than the $Rn^t$ grains, and relatively many plants with a green midrib are the result. That no such plants appear in crosses where $Rn^t\ Rn^t$ is the female parent could largely be explained as a result of the absence of such a concurrence in the female sex.

A third case of fragmentation of a genetically marked chromosome is known from the crosses of HIORTH with *Whitneyi* plants having the dominant gene $C^{pa}$ (HIORTH, 1946 c). $C^{pa}$ causes certain patterns on the cotyledons, their base having a reddish violet colour and a red arrow appearing higher on the cotyledon. Very young leaf buds are also reddish. Most $C^{pa}$ are monosomic complex heterozygotes $a \,.\, g$ having the pairing 4 II + I + chain-of-four, $a$ being the standard complex of *G. Whitneyi*, $g$ being a complex with only 6 chromosomes. $C^{pa}$ has its locus in a $g$ chromosome of the chain, and the frequent elimination of the I, which is an $a$ chromosome, results in a great excess of $C^{pa}$ in crosses $C^{pa} \times c\ c$. There is, however, a disomic $C^{pa}$ with the pairing 5 II + chain-of-four that showed a normal segregation of $C^{pa}$ in female backcrosses. Now, a third $C^{pa}$ type has been found which in such crosses gives only few $C^{pa}$ plants. This type is trisomic with the pairing 6 II + III or 7 II + I, and no doubt is $a \,.\, a +$ the $C^{pa}$ chromosome. It must have arisen through a non-disjunction in a monosomic, the $C^{pa}$ chromosome having followed the $a$ chromosomes of the chain. Four $C^{pa}$ plants in the offspring of a cross with this new type were investigated. Three plants showed an extra chromosome of normal size; in one, however, there was only a fragment chromosome about $^1/_4$ the size of the normal chromosome (Fig. 5 *f*). In most p.m.c's the fr. chromosome was unpaired (Fig. 5 *g*), though it could form a hetero-III with two normal chromosomes (Fig. 5 *h*), but no hetero-II was observed. These facts make it probable that the small chromosome is a fragment of the $C^{pa}$ chromosome still containing the gene $C^{pa}$. Elimination of the small chromosome was not infrequent at anaphase 1.

HIORTH has had progenies of the plants that had been studied

cytologically. The fragment plant showed a better pollen and seed fertility than the plants with a normal $C^{pa}$ chromosome, although the segregation was very similar to that of the latter plants. This was perhaps unexpected: ». . . man sollte annehmen, dass Gameten mit Fragment besser funktionstüchtig sind, als solche mit einem ganzen überzähligen Chromosom. Dies dürfte indessen durch eine höhere Elimination des Fragmentes ausgeglichen werden» (HIORTH). The family originating from the fragment plant could only be studied by HIORTH in the seed bed. $C^{pa}$ plants here showed a striking peculiarity. The cotyledon pattern was more marked than usual, and all seedlings had one or more green sectors surrounded by reddish-violet tissue. One seedling had a violet and a green cotyledon. In the three sister families having a normal $C^{pa}$ chromosome no seedling showed any green sector. These facts seem to prove that it was the $C^{pa}$ chromosome that had been fragmented. The green sectors must have arisen through somatic elimination of $C^{pa}$. The fragment chromosome must be rather frequently eliminated at mitosis, while a normal $C^{pa}$ chromosome is not eliminated. A study of the process of elimination was not attempted because no fixation of root tips could be procured. Thus, the cause of this different behaviour of the changed $C^{pa}$ chromosome cannot be discussed.

## CONCLUSIONS.

Several cases of spontaneous chromosome changes have been found during cytological investigations of the $F^{b}$ plants in the successive backcross generations, others have been detected after an investigation of material that had shown an aberrant genetical behaviour. In the species cross a change had once been brought about through a reciprocal translocation between the larger end-chromosome of the heterochain and a chromosome not belonging to this chain; probably an inversion had also occurred. Investigation of the genetically aberrant material has shown sure cases of fragmentation: in $Rn^{t}\,Rn^{t}$ the translocated foreign segment is comparatively often lost, while the small fragment chromosomes in *Kl* and trisomic $C^{pa}$ are also the result of fragmentation. On the other hand, the variation in size of the chromosome 2.3' in the species cross was more consistent with the view that it was the result of exchanges between the chromosomes in a hetero-III.

A few examples of spontaneous chromosome changes that have arisen in species crosses may be given. SVESHNIKOVA (1936) investigated

*Vicia sativa* ($n = 6$) × *V. amphicarpa* ($n = 5$). $F_1$ sometimes formed a hetero-III, the small *F* chromosome from *sativa* being paired with the *A* chromosomes from the two species. In later generations new chromosomes were observed. One of the $F_2$ plants had two »unusual» chromosomes, one of them a very small *F*, the other an enlarged *A*. The new chromosomes must have been formed through an interchange between »non-homologous» chromosomes in the III. Plants in later generations had only one of the changed chromosomes and none of them was homozygous for any of these chromosomes, indicating homozygous lethality. KOSTOFF (1939) found in later generations of amphidiploid *Nicotiana glauca* × *N. Langsdorfii* one $F_3$ plant and one $F_4$ plant with new chromosomes. He is of the opinion that the new chromosomes have arisen through unequal crossing-over, multivalents being frequent. TOBGY (1943, p. 103) mentions the occurrence of new chromosomes in $F_2$ of *Crepis neglecta* × *C. fuliginosa*. The pairing in $F_1$ was good but paired chromosomes were very different in size or form; hetero-III's and hetero-IV's were frequent.

The interesting studies of WESTERGAARD (1946) on aberrant forms of *Melandrium* have given examples of chromosomes changed through fragmentation or through exchange of segments. WESTERGAARD (cf. also WARMKE, 1946) has found three different aberrant *Y* chromosomes in the offspring of triploid *Melandrium*. One was a terminal fragmentation of the so-called differential arm of the *Y* chromosome, one was a fragmentation in the homologous arm, that is, the arm that forms a chiasma with the *X* chromosome. The third aberrant *Y* was the result of an exchange between the differential arms of *Y* and *X*. Plants having aberrant *Y* chromosomes are intersexual or male sterile.

The frequency of spontaneous chromosome changes is no doubt difficult to estimate. If they are accompanied by phenotypical changes, as in *Melandrium* or *Kl* and $Rn^t$ in *Godetia*, they have a great chance of being detected, if not, the chance is small unless, as in species crosses, many plants are investigated. But even if one takes into consideration these facts, it cannot be doubted that exchanges between chromosomes which are structurally different are the most important cause of the appearance of chromosomes of a new size or form; it seems that such exchanges are facilitated in hetero-multivalents.

The new chromosomes may have unusual properties. In $Rn^t\,Rn^t$ a *Whitneyi* chromosome having a translocated *deflexa* segment shows a relatively high frequency of spontaneous changes; the foreign segment is rather often lost. The fragment chromosome carrying $C^{pa}$ is

often eliminated at mitosis, as was shown by green sectors on the seedlings. The small chromosome that had arisen through a fragmentation of the chromosome carrying *G* also seems to be eliminated at mitosis.

## SUMMARY.

The cytological investigation of *Godetia* plants from the cultures of Dr. GUNNAR HIORTH, Vollebekk, Norway, has shown several cases of spontaneous chromosome changes.

In two cases chromosome fragmentation explains the unexpected appearance of the recessive character in heterozygotes, a segment with the dominant allele being lost. A *Whitneyi* chromosome with a *deflexa* segment is rather often fragmented, the foreign segment being lost. A fragment chromosome carrying $C^{pa}$ is often eliminated at mitosis.

Dr. HIORTH tried to transfer the petal spot $F^b$ from *G. amoena* to *G. Whitneyi* by means of backcrossing for successive generations the $F_1$ hybrid to *G. Whitneyi.* All $F^b$ plants in the first backcross generation $F'_2$ were very sterile, and their chromosome pairings showed the presence of several *amoena* chromosomes. Later generations showed different $F^b$ types. The heterotrisomic type appeared for the first time in $F'_4$ and had a certain amount of sterility and 6 II + heterochain-of-three. It had 13 *Whitneyi* and 2 *amoena* chromosomes, i. e. the end-chromosomes of the chain. The larger end-chromosome is the $F^b$ chromosome, but it must be supplemented by the small end-chromosome to give a fertile gone. One trisomic plant had a chain or III consisting of the $F^b$ chromosome and two *Whitneyi* chromosomes. Still more sterile is a disomic $F^b$ type. This must have at least three *amoena* chromosomes, for it forms the heterochain and a second configuration-of-three. Exchanges in the heterochain are probably the explanation of the fact that the small chromosome changes its size; in one family it was particularly small. The greater constancy of the heterochain in later generations is perhaps also a result of exchanges. Of rare occurrence is a fertile $F^b$ type. It has 7 II and a regular meiosis. Here, $F^b$ must have been transferred through crossing-over to the *Whitneyi* chromosome in the centre of the chain. Thus, the petal spot $F^b$ is transferred to the species *Whitneyi* by a rare crossing-over in a heterochain. The gene $C^{sp}$ from *amoena* is also followed by sterility in similar crosses, and meiosis in an $f\,C^{sp}$ plant in $F'_2$ showed the presence of a homochain, indicating chromosomal sterility.

## LITERATURE CITED.


1. CHITTENDEN, R. J. 1928. Notes on species crosses in *Primula, Godetia, Nemophila* and *Phacelia.* — Journ. of Genetics 19.
2. HIORTH, G. 1940. Eine Serie multipler Allele für Blütenzeichnungen bei *Godetia amoena.* — Hereditas XXVI.
3. — 1942. Zur Genetik und Systematik der *amoena*-Gruppe der Gattung *Godetia.* — Zschr. f. ind. Abst.- u. Vererbungslehre 90.
4. — 1946 a. Über drei allotrisome Typen sowie eine interspezifische Translokation aus *Godetia Whitneyi* × *G. deflexa.* — MS.
5. — 1946 b. Zur Genetik des Artbastardes *Godetia amoena* × *G. Whitneyi.* — MS.
6. — 1946 c. Zur Genetik der Monosomen von *Godetia Whitneyi.* — MS.
7. HÅKANSSON, A. 1931. Chromosomenverkettung bei *Godetia* und *Clarkia.* — Ber. d. deutsch. botan. Gesellschaft 49.
8. — 1940. Die Meiosis bei verschiedenen Mutanten von *Godetia Whitneyi.* — Lunds Univ. Årsskrift. N. F. Avd. 2, Bd. 36, Nr. 5.
9. — 1941. Zytologische Beobachtungen an Kreuzungen zwischen *Godetia deflexa* und *G. Whitneyi.* — Botan. Notiser.
10. — 1942. Zytologische Studien an Rassen und Rassenbastarden von *Godetia Whitneyi* und verwandten Arten. — Lunds Univ. Årsskrift. N. F. Avd. 2, Bd. 38, Nr. 5.
11. — 1943. An interspecific translocation causing changed segregation ratios. — Hereditas XXIX.
12. — 1944. Eine »homozygotische» interspezifische Translokation. — Hereditas XXX.
13. — 1946. Meiosis in hybrid nullisomics and certain other forms of *Godetia Whitneyi.* — Hereditas XXXII.
14. KOSTOFF, D. 1939. Studies on polyploid plants. XXI. Cytogenetic behaviour of the alloploid hybrids *Nicotiana glauca* GRA. × *Nicotiana Langsdorfii* WEINB. and their evolutionary significance. — Journ. of Genetics 38.
15. RASMUSON, H. 1921. Beiträge zu einer genetischen Analyse zweier *Godetia*-Arten und ihrer Bastarde. — Hereditas II.
16. SVESHNIKOVA, I. N. 1936. Translocations in hybrids as an indicator of »karyotype evolution». — Biologichej Zhurnal 5.
17. TOBGY, H. A. 1943. A cytological study of *Crepis fuliginosa, Cr. neglecta* and their $F_1$ hybrid, and its bearing on the mechanism of phylogenetic reduction in chromosome number. — Journ. of Genetics 45.
18. WESTERGAARD, M. 1946. Aberrant *Y* chromosomes and sex expression in *Melandrium album.* — Hereditas XXXII.
19. WARMKE, H. E. 1946. An analysis of male development in *Melandrium* by means of *Y* chromosome deficiencies. — Abstracts of papers presented at the delayed 1945 meetings of the Genetics Society of America; Genetics 32.

# HETEROCHROMATIC B-CHROMOSOMES IN ANTHOXANTHUM

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## I. INTRODUCTION.

IN a preliminary note by the present writer (1942) on the chromosome numbers in the grass genus *Anthoxanthum* it was reported that some plants of *A. aristatum* had, in addition to the normal 10 chromosomes, also 2—3 extra »fragments».

The population in which this was found was more thoroughly investigated later on, and the results thus obtained will now be described. The possibility suggested by ÖSTERGREN (1945) that such extra chromosomes lead a kind of *parasitic existence* in the populations will also be given a renewed analysis in this connection.

There are many names which have been given to such supernumerary chromosomes as are not homologous with those of the normal complement and as exist in equilibrium in the populations. Naturally, every investigator is at liberty to adopt a name of his own choice for those extra chromosomes which he has discovered. I have »baptized» them *B-chromosomes* in my *Anthoxanthum* case. The reason for this is that they probably constitute a somewhat related case to the supernumerary chromosomes of maize, which are well-known to all geneticists under this name. DARLINGTON and THOMAS (1941) have also adopted this name for the heterochromatic extra chromosomes studied by them in *Sorghum purpureo-sericeum*. The name »accessory chromosomes» suggested by MÜNTZING (1945) and HÅKANSSON (1945) and also used previously in the literature (e. g., COLEMAN, 1943, p. 7) is also a suitable term. »Extra fragment chromosomes», however, is undoubtedly a term which is not appropriate at all for such cases as the present one, as it easily leads to the misunderstanding that the chromosomes have originated from the normal complement by simple fragmentation, a view that can be demonstrated to be wrong.

## II. MATERIAL AND METHODS.

The species of the genus *Anthoxanthum* are typical cross-fertilizers, a fact demonstrated by (1) their flower biology (protogyny), (2) the

high variability of single plant progenies, and (3) the poor seed formation on isolation.

The material of *Anthoxanthum aristatum* containing these B-chromosomes was received from the Botanical Garden of Coimbra, which had collected the seeds on wild plants in Portugal. A comparison of my plants with the descriptions of the Portuguese *Anthoxanthum* species by COUTINHO (1939) shows that they undoubtedly belong to the species *A. aristatum* BOISS.

During the study of meiosis in the plants with B-chromosomes it was found of value to compare the behaviour of B-univalents with that of univalent chromosomes of the normal complement. Such univalents (from non-pairing in normal diploids) are occasionally found in plants of the same material, but to get a bigger material of normal univalents *trisomic* plants of another *Anthoxanthum* population were studied. In material of *Anthoxanthum aristatum* from the Botanical Garden of Nancy (France) an autotriploid plant was obtained in the progeny of acenaphthene-treated material. This triploid was crossed with diploids of the same population and in this way trisomic plants were obtained.

This *Anthoxanthum* population from Nancy is morphologically very different from the Coimbra material, but the two types are easily crossed and give a hybrid of good fertility, so it is likely that they belong to the same species. This taxonomic question, however, although not very important in the present connection, should be analysed more thoroughly before definite statements are made in this respect. Anyhow, the two types are closely related, and a comparison is therefore justifiable.

The root tips were usually fixed in chrome-acetic-formalin (the NAVASHIN modification by MÜNTZING, 1933, p. 131). For chromosome morphological study root tips of a few plants were fixed in LEVITSKY's fixative (10 % formalin + 1 % chromic acid, equal parts). For study of meiosis the panicles were fixed in chrome-acetic-formalin after prefixing in acetic alcohol (1 : 3). The microtome sections of panicles and root tips were stained with crystal violet or (in a few cases) by the FEULGEN method. The first pollen mitoses were studied in acetocarmine smears and also in material fixed in acetic alcohol, sectioned on a microtome and stained with FEULGEN.

## III. CYTOLOGICAL RESULTS.

### 1. CHROMOSOME MORPHOLOGY.

A few fixations of root tips from the Coimbra material were made in LEVITSKY's fixative in order to give information concerning the chro-

mosome morphology. Measurements of the chromosomes by the usual method (LEVITSKY, 1931) were undertaken on a slide with $2n = 10 + 2$ B. Seven metaphase plates were measured. The chromosomes of the normal complement apparently belong to only two morphologically different types; in the haploid set of five chromosomes there are three with a secondary constriction and two without. The result of these measurements is given schematically in Fig. 2, where a B-chromosome is also depicted. Fig. 1 is a LEVITSKY-fixed metaphase from a plant with $2n = 10 + 3$ B. The B-chromosomes in this material belong either to the »standard type» or to the »iso-chromosome type» (cf. below). Which of these types they actually belong to cannot be determined, as only root tips were seen of these plants. The chromosomes of the normal set are about 6 $\mu$ and those of the B type about 4 $\mu$ in length. Of course, it is very likely that the three chromosomes with the secondary constriction are in reality slightly different from one another in morphology and that the same is the case with the other type, but these differences are too small to be ascertained.

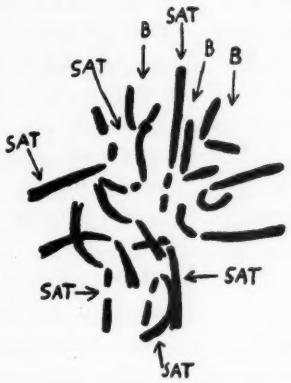



Fig. 1, *A. aristatum*, root-tip mitosis in a plant with $2n = 10 + 3$ B. At least 4 of the 6 secondary constrictions are of SAT-type. All chromosomes with secondary constrictions, however, have preliminarily been marked SAT in the figure. Fixative: LEVITSKY. — × 3000.

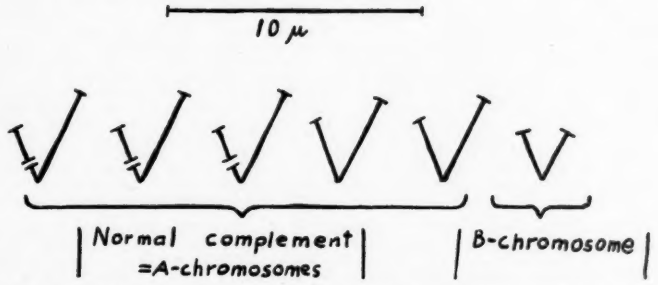



Fig. 2, scheme of the morphology of the haploid chromosome set plus a B-chromosome in *A. aristatum*.

At least two of the three chromosomes with secondary constrictions are SAT-chromosomes, concerned with the formation of nucleoli, as

sometimes 4 nucleoli have been seen in the root-tip nuclei. As, however, nucleoli have a strong tendency to fuse, it is quite possible that all three of them are of SAT-type, and all the chromosomes with secondary constriction have preliminarily been marked »SAT» in Fig. 1.

The numerous fixations in chrome-acetic-formalin, although less clear in this respect, give definite support to the above chromosome morphological scheme.

The chromosome morphology of the Nancy type has not been analysed in detail, but so much is obvious that it is clearly different from the Coimbra material.

## 2. FREQUENCY AND TYPES OF B-CHROMOSOMES.

The *Anthoxanthum* population from Coimbra contained B-chromosomes of the following three types:

(1) *»Standard» type.* — These chromosomes are about 4 $\mu$ in length at mitosis and their two arms are of the same length.

(2) *Iso-chromosomes.* — These are morphologically quite similar to the preceding type, but they differ from it in their behaviour during meiosis, where their two arms are able to pair with one another, thus giving ring-shaped univalents.

(3) *Very small chromosomes.* — These have only been seen in meiosis, where they appear as small round dots (Figs. 25—27).

The two first types are rather similar to one another. To distinguish between them requires a careful study of meiosis, and in cases of very strong chromosome contraction even this may not be sufficient, as then the rod univalents may also appear to be round like the rings of iso-univalents. (The chromosome contraction at meiosis is highly variable in this material.) Consequently, it is not possible always to state to which of these two types a B-chromosome belongs.

The standard type seems to be the commonest one. That is why I have called it standard. But it may of course be possible that a study of more extensive material will show that some other type is even more common.

The chromosomes of the third type have been very little studied, so all descriptions below will refer to the first two types, unless otherwise is stated.

The frequency of the various types of B-chromosomes was studied in meiosis of plants derived from the original seed sample, and the result was as follows:

*0 B*: 12 plants.

*1 B*: 4 plants. Two of them have standard B's and the two others have B's of the very small type.

*2 B*: 2 plants. Both have standard B's. One of them had 2 B's in all studied parts, the other was a chimaera having 1 B in one flower and 2 B's in another.

*3 B*: 1 plant. It seems that all chromosomes are of standard type.

*4 B*: 3 plants. One seems to have all four chromosomes of the standard type; the second has at least two of iso-type, the rest of them may be iso- or standard chromosomes; the third has at least one iso-chromosome + two of iso- or standard type and its fourth chromosome of the very small type.

Thus, out of 22 plants there were 10 which had one or more B-chromosomes. It may, however, be a little dubious whether this determination is a reliable estimate of the frequency of B-chromosomes in the wild population. Firstly, of course, the number of plants studied is rather small, and, secondly, nothing is known as to whether the seed sample had been collected from many plants or perhaps only from a single one.

However, it is rather likely that they are common. If they were not, there should have been only a small chance of getting, as I did, three different types in so small a sample.

### 3. HETEROCHROMATY OF THE B-CHROMOSOMES.

As the accessory chromosomes of some plants are known to be heterochromatic (e. g., *Zea mays*, *Sorghum purpureo-sericeum* and *Narcissus Bulbocodium*), it was of some interest to see whether this is the case in *Anthoxanthum* as well. My first impression was that it was not, but later on I got clear evidence of the heterochromatic nature of the accessory chromosomes in the present material.

There are some known facts and hypotheses concerning the genetic and physiologic properties of heterochromatin, but it should not be forgotten that heterochromatin is primarily a *morphological* concept, as already pointed out by HEITZ (1928, p. 765) in his original definition of heterochromatin.

Here the slightly improved definition of HEITZ (1932, pp. 626—627) may be given: »Als Heterochromatin bezeichne ich also ein in der Grundsubstanz des Ruhekerns sichtbar vorhandenes, in Bezug auf die Längsrichtung des Chromosoms bestimmt gelegenes Stück desselben, welches mindestens in der Telo- und Prophase,

manchmal auch in der Ana- und Metaphase stärker färbbar ist als die übrigen Stücke, als das die Grundstruktur bildende Euchromatin.»

A fact already recognized in HEITZ's original paper (1928) should also be pointed out, viz. that the heterochromatic behaviour may sometimes be manifested in all nuclei or divisions and that in other cases it may be restricted only to special phases in the life cycle of an organism.

Later on, it turned out that chromosome parts which exhibit overstaining (positive heteropycnosis) at some stage, and thus are in agreement with HEITZ's definition, may still differ from this definition by manifesting an *understaining* (negative heteropycnosis) at some other stage. (The terms positive and negative heteropycnosis are due to WHITE, 1935, p. 66.)

A common view of what heterochromatin is may now be expressed in the words of WHITE (1945): »It will be convenient to use the term *heterochromatic* to describe any chromosomal region which becomes heteropycnotic at some stage in its cycle. Chromosomal regions which never under any circumstances show heteropycnosis may be called *euchromatic*» (l. c., p. 28).

The recognition that negative heteropycnosis should also be ranked among the heterochromatic phenomena may very well make the groups »heterochromatin» and »secondary constrictions» confluent. (Cf. also the speculations of KLINGSTEDT, 1941, pp. 171 and 173.)

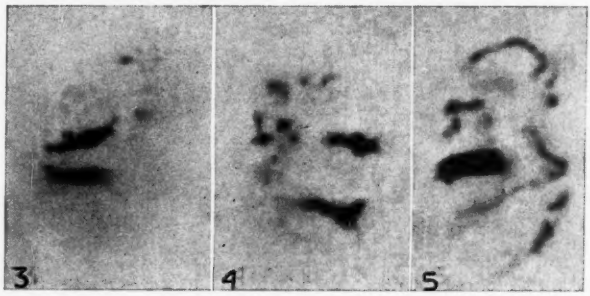
The heterochromatin of my *Anthoxanthum* material has only been studied in the root tips. The normal complement of *Anthoxanthum aristatum* also contains some heterochromatin, forming a number of small overstained bodies in the resting nuclei. In rare cases these may by chance have been brought in a position close to one another, so that they together form a somewhat larger body. In the plants with B-chromosomes, however, larger heterochromatic bodies of this kind are much more common, and they increase in frequency with an increasing number of B's in the plant. It would be expected that these big chromocentres would be equal in number to the B's of the plant; and cells having that number of chromocentres can also be found (e. g., Figs. 3—4). In most cells of plants having more than one B, the number of chromocentres is, however, lower than expected. This is undoubtedly due to a close association of B's with one another, whereby they form joint chromocentres.

In the root-tip prophases the change of these chromocentres into mitotic B-chromosomes can be followed. In the prophases, too, they often show a stronger staining than the normal complement. Furthermore, it can be seen here that the B's quite often lie very close to one another (Fig. 5), an observation that confirms the above view as to the reason the chromocentres in the resting nuclei are often fewer in number than the B-chromosomes at metaphase.

This type of association can also frequently be found within a single chromosome, its two arms associating with one another.

Such a close association of heterochromatic parts is a fact also known from many other organisms. A well-known example is the chromocentre in the salivary gland nuclei of *Drosophila*. GEITLER (1939) also found that the heterochromatic *Y*-chromosomes in various *Heteroptera* often formed a joint chromocentre in polysomatic nuclei. Fusion of heterochromatic bodies in *Fritillaria* was reported by DARLINGTON and LA COUR (1941).

The degree of heteropycnosis in the resting nuclei is very variable in my material. In some plants it is much more pronounced than in others. Furthermore, there is a very characteristic difference between cells in the same root tip. The heterochromatin is much more easily



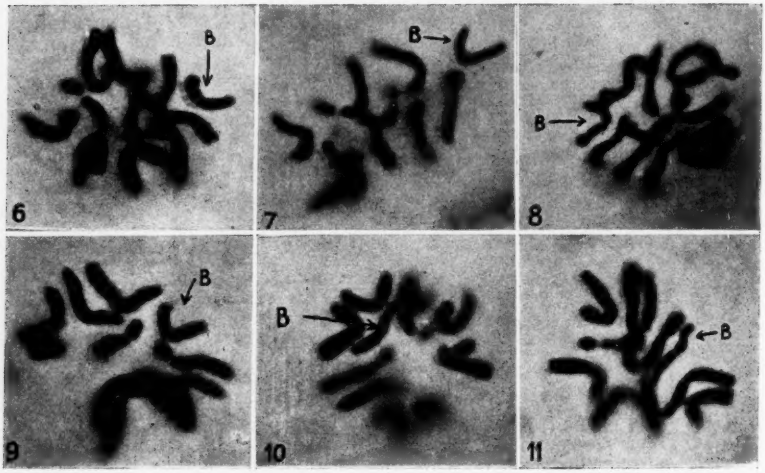

Figs. 3—5, positive heteropycnosis of B-chromosomes in root-tip cells of a plant with 2 B's. — Figs. 3—4, resting nuclei. — Fig. 5, prophase. — × 2700.

seen in somewhat older cells (in the higher regions of the root) than in the young vigorously growing meristematic cells.

This difference is not only due to the fact that the euchromatin is more weakly staining in the older cells, but it actually also seems that the B-chromosomes are more diffuse and weakly staining in the resting nuclei of the younger cells than in the older ones.

The B-chromosomes also show a differential pycnosis at metaphase, but then the difference is of the opposite kind, they are narrower than the normal chromosomes (negative heteropycnosis). This is especially clear in cold-treated plants (Figs. 9—11) but a slight difference of this type also exists at normal temperature (Figs. 6—8). It might be suggested that the more pronounced negative heteropycnosis at low temperature is due to a »differential reactivity» of the same type as that found by DARLINGTON and LA COUR (1938, 1940, 1941) for seg-

ments of the normal chromosomes in *Paris*, *Trillium* and *Fritillaria*, by GEITLER (1940) in *Adoxa*, by LEVAN (1942) in *Secale*, and by CALLAN (1942) in *Triton*. The negative heteropycnosis in my case, however, seemed to be visible even at normal temperature, and it is possible that its clearer visibility at low temperature may be due simply to the fact that differences in width are more easily seen when the absolute width of normal as well as B-chromosomes is larger because of the cold contraction. Hence it need not necessarily mean a differential reaction to low




Figs. 6—11, negative heteropycnosis of B-chromosomes in root-tip mitoses of a plant with one B at different temperatures. — Figs. 6—8, ordinary temperature (19° C. at the time of fixation). — Figs. 9—11, cold treatment for four days at — 1,5° C. — × 2450.

temperature, even if this is a likely interpretation. It should also be pointed out that the negative heteropycnosis is rather variable in my material (like the positive one) and that it is not always easily seen.

There are other cases known of negative heteropycnosis at normal temperature, especially in animals (WHITE, 1935, 1940; GEITLER, 1937; KLINGSTEDT, 1941; and KOLLER, 1938). LEVAN (1942) found that the differential segments of rye which could be made visible at metaphase by cold treatment could also be found at normal temperature in a *haploid* rye plant, and he concludes that the lower viability of the haploid obviously acts here in the same manner as cold treatment (l. c., p. 182).

The B-chromosomes of *Anthoxanthum* do not seem to be heteropycnotic during the meiotic prophase.

It is not known whether there is a difference in heteropycnotic behaviour between the B-chromosomes of standard type and those of iso-type in my material.

## 4. CONSTANCY OF THE B-CHROMOSOMES.

Some cases are known where the number of accessory chromosomes is changed during the development of the individual. Firstly, there are *Sorghum purpureo-sericeum* (DARLINGTON and THOMAS, 1941) and a type of *Poa alpina* (MÜNTZING, 1946 b), in which plants the accessory chromosomes are regularly eliminated from the somatic tissues of the individuals (or at least their root tips) and only preserved in the germ-track. Secondly, there are cases where the accessory chromosomes seem to show a more general instability in number. This is known, e. g., in *Tulipa galatica* (DARLINGTON, 1937, p. 63, referring to unpublished results of UPCOTT). The B-chromosomes of maize also exhibit occasional changes in number during the development of the plants (DARLINGTON and UPCOTT, 1941, p. 279). In this case it is also possible directly to see the aberrant behaviour of the B-chromosomes during the mitoses. They usually lie at the edge of the metaphase plate and their centromeres may divide either before or after those of the normal chromosomes (DARLINGTON and UPCOTT, l. c.). This is regarded as due to a centromere weakness.

My observations on the *Anthoxanthum* material demonstrate that the B-chromosomes of standard and iso-type (which must be treated together, as they are indistinguishable in many cases) have rather a good constancy in the various parts of the plants; changes in number do occur but are relatively rare.

Thus, in 86 plants in which the somatic number of B's (1—4) was determined, counts were made (in practically all cases) in at least three different roots of the same plant. These three roots gave the same number in all cases with the single exception of an entirely tetraploid root where the number of A- as well as B-chromosomes was doubled. This exception, of course, is not due to aberrant behaviour of the B's. Nor were there ever seen in these mitoses any signs of abnormal behaviour of the B's, such as precocious or delayed centromere division or a delayed congression on the plate.

In 31 plants (13 with no B in the roots and 18 with one or more

B's) the same number of B's was found in both root tips and meiosis. In two plants, however, the number of B-chromosomes differed between root tips and anthers, and in a third case there was a difference between different flowers in the same panicle, one flower having a single B, another having two.

The B-chromosomes of the very small type seem to be more irregular. They show disturbances of congression and probably also of division at the second division of meiosis, and in all the three cases in which root tips and anthers were compared these B's had been eliminated from the root tips. Anyhow, these very small B's have been found to be transmitted from one generation to the next (observed at meiosis in mother and progeny), so they must have a certain degree of stability.

### 5. BEHAVIOUR AT MEIOSIS.

The chromosomes of the normal complement in *Anthoxanthum aristatum* usually pair as $5_{II}$ at meiosis. There is often a low frequency of non-pairing in the plants, as is probably found in most organisms. Multivalents have not been found in the about 20 plants examined in more detail. (In this respect this diploid species differs strikingly from the tetraploid *A. odoratum*, where multivalents, even such higher than quadrivalents, are of quite common occurrence; KATTERMANN, 1931; PARTHASARATHY, 1939; and ÖSTERGREN, 1942.)

The behaviour of *normal* chromosomes in excess has been studied in triploid and trisomic plants of the material from Nancy. These chromosomes often form trivalents but are also often left unpaired as univalents (Figs. 12 and 13).

During anaphase of the first division these univalents often lag, and the lagging univalents very frequently divide into two daughter univalents, which are usually included in the telophase nuclei (Fig. 14). In the second division these daughter univalents are unable to divide and they lag between the separating anaphase chromosomes (Fig. 15). In this way they are very often left outside the four normal tetrad nuclei, and form separate micronuclei (Fig. 16), which at later stages can be seen to transform into dark-staining chromatin droplets. Thus, there is a considerable elimination of univalents derived from the normal complement.

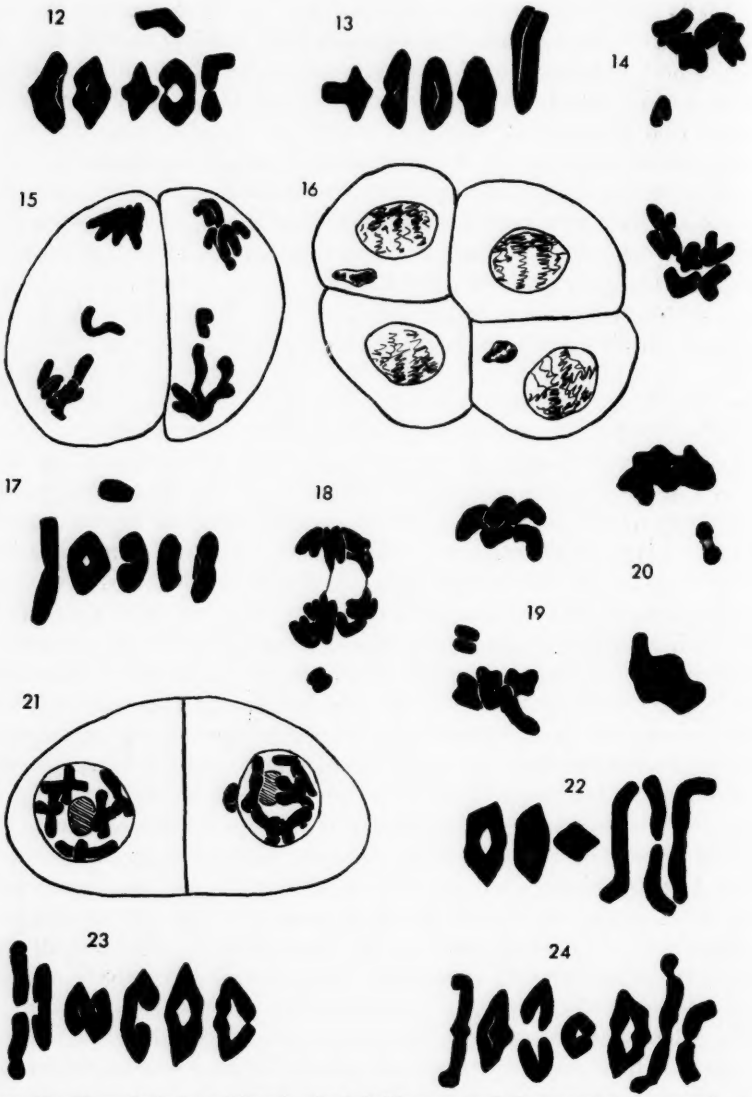
The behaviour of univalent B-chromosomes is quite different from this. They can be studied quite well in plants having a single B-chromosome (of »standard» type).

The extra B-chromosome does not pair with the normal chromosomes. In a plant of this type 208 cells were studied; in 207 of them the B was unpaired and in a single one it was in terminal contact with a rod bivalent, probably forming a trivalent. A great number of other first metaphases were also studied in another plant (without counting them) and the B-chromosome was always unpaired. The single case of pairing with a normal chromosome is not sufficient to be made the basis of a hypothesis of partial homology of the B with this chromosome. It may very probably be a quite unspecific sticking together of heterochromatic parts.

At anaphase of the first division the B-univalents, like the normal univalents, may either be included in one of the polar groups or may lag between them (Figs. 18—19). Here, however, there is a very important difference from the normal univalents. *Lagging univalents of B-type never divide at first anaphase.* They manifest their doubleness (i. e. that they consist of two chromatids) like all the other chromosomes at this stage; a result considered to be due to »lapse of chromatid attraction». But the two chromatids are never found in a stage of moving apart, as is so often observed in normal univalents. The centromere can sometimes be seen to be subjected to tension in the direction of the two opposite poles, but there is some *connecting link* (either in the centromere itself or very close on both sides of it?) which keeps the two halves together. This link is seen to be extended (Fig. 20), but it does not break, so there will be no effective division. Such a lagging B-chromosome may later (in early telophase) be included in one of the two normal nuclei, or it may form a small nucleus by itself, often in close proximity to one of the big nuclei (Fig. 21).

As a consequence of the absence of division at first anaphase, the B-chromosome is able to divide normally in the second division. In the tetrads micronuclei formed by eliminated B-chromosomes are practically never seen. Not a single one was found in 219 tetrads. In a slide from a trisomic plant, on the other hand, a count among 132 tetrads showed 79 with one or more micronuclei and only 53 without — a striking difference.

The non-division of univalent B-chromosomes at first anaphase, whereby they differ from the univalents of the normal complement, appears as *a purposeful adaptation to maintain the B-chromosomes in the population* (cf. below). The case also tells us that a higher frequency of unpairing does not necessarily mean a higher elimination of




Figs. 12—16, meiosis in a trisomic plant (with a *normal* chromosome in excess). — Fig. 12, first metaphase, $5_{II} + 1_{I}$. — Fig. 13, first metaphase, $4_{II} + 1_{III}$. — Fig. 14, first anaphase with a dividing univalent (the lower daughter univalent has reached the anaphase group). — Fig. 15, lagging daughter univalents at second anaphase. — Fig. 16, a tetrad with micronuclei formed by daughter univalents.

a chromosome, and thus warns us not to make hasty conclusions in this respect.

In plants with two B-chromosomes of »standard» type these very regularly pair with one another, forming a small bivalent, usually of the ring-shaped type with chiasmata at both ends (Fig. 22). In 54 cells of a plant of this type the frequency of chiasmata at metaphase in the B's was compared with that in the normal chromosomes. The average for the B's was 1,833 per bivalent and that for the normal chromosomes 1,737. The difference is not statistically significant ($P > 0{,}2$).

The B-chromosomes never had more than a single chiasma in the same chromosome arm, while the normal chromosomes sometimes had two. If, instead of counting the exact frequency of chiasmata, we only count the frequency of paired chromosome arms, a procedure sometimes adopted in such investigations, we shall thus get a different result, viz. 1,833 for the B's and 1,596 for the normal bivalents. Measured by this method the pairing is significantly higher in the B's ($P < 0{,}01$).

The good pairing of the B's was confirmed (although not quantitatively analysed) in another plant with two B's of standard type. The good pairing of the B-chromosomes is rather surprising; firstly, because they are shorter than the normal chromosomes, and, secondly, because they are heterochromatic. The heterochromatic B-chromosomes of maize show a reduced pairing as compared with the normal chromosomes (DARLINGTON and UPCOTT, 1941). Perhaps the good pairing of the B's in the present case is connected with the absence of heteropycnosis in them at the prophase of meiosis. In tetraploid spermatocytes of a locust WHITE (1933) found that the two *X*-chromosomes were still heteropycnotic, just as the single *X* in the normal diploid spermatocytes. These heteropycnotic *X*-chromosomes were unpaired. In the female meiosis, however, where the two *X*'s are not heteropycnotic or only so to a small degree, they show a good pairing. WHITE concluded that the absence of pairing in the males was due to the heteropycnosis (cf. also WHITE, 1945, p. 89, and COLEMAN, 1943).

Figs. 17—24, meiosis in various plants with B-chromosomes. — Fig. 17, first metaphase, $5_{II} + 1\,B_I$. — Figs. 18—20, first anaphases in a plant with a single B. — Fig. 18, the univalent has a polar position and will no doubt be included in the anaphase group. — Fig. 19, a lagging B-univalent manifests doubleness but does not divide. — Fig. 20, like the previous figure, but a later stage, a connecting link prevents the chromatids from moving apart (univalent seen in end view). — Fig. 21, prophase of second division, the undivided B has formed a small nucleus close to one of the big nuclei. — Fig. 22, first metaphase, $5_{II} + 1\,B_{II}$. — Fig. 23, first metaphase, $5_{II} + 1\,B_{IV}$. — Fig. 24, first metaphase, $5_{II} + 2\,B_{II}$. — × 2650.

Thus, there is some evidence that the absence of heteropycnosis in a heterochromatic chromosome may favour its pairing.

Consistent with this good pairing of the B-chromosomes is also the fact that they quite commonly form trivalents and quadrivalents in the plants having three and four B's. The frequency of trivalents in the 3 B plant studied is about as high as in plants having three homologous members of the normal complement (trisomics and a triploid), i. e. about 50 %.

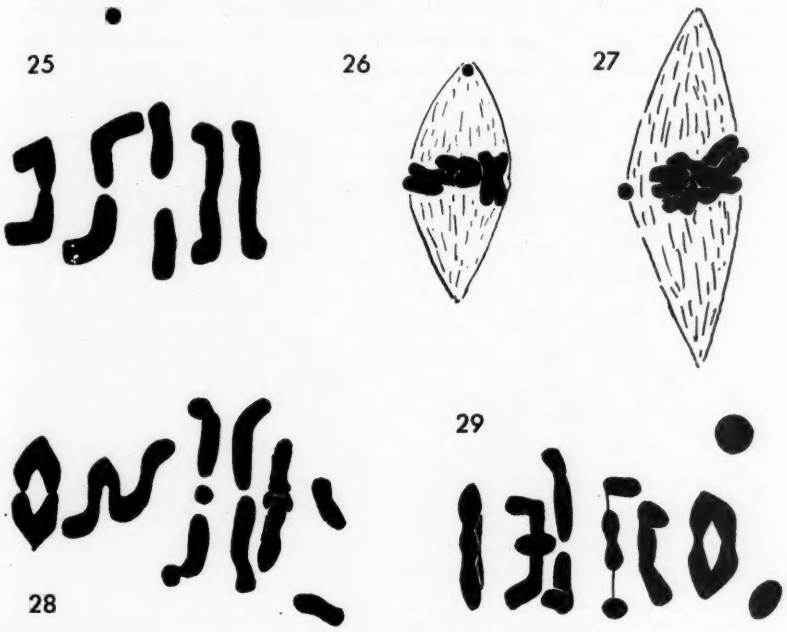
This observation is important when we have to consider the homology relations of the B-chromosomes in *Anthoxanthum*. We know that the normal chromosomes have a good ability to form trivalents among themselves when they are three and that the same is the case with the B-chromosomes, and, further, that the B-chromosomes do not pair with the normal chromosomes, not even in plants having only a single B. The conclusion must be that they are not homologous with the chromosomes of the normal complement. (The single cell, out of 200, where there might have been such a pairing between A- and B-chromosomes does not mean very much in this discussion.)

*Iso-chromosomes.* — In one 3 B plant I found that at least one chromosome was an iso-chromosome, there being seen in the univalents a pairing between the two arms of the same chromosome to form rings. Similarly it was found that one 4 B plant must have at least two such iso-chromosomes. Especially this 4 B plant gave very clear evidence in this direction (Fig. 29). These two iso-chromosome plants were also characterized by a higher frequency of univalent B-chromosomes, as should be expected from the fact that, here, an intrachromosomal pairing is competing with the normal interchromosomal one.

It might be suggested that all the B's of this size (groups 1 and 2, see p. 264) might be iso-chromosomes, but this is very unlikely when we consider the very perfect interchromosomal pairing in the 2 B plants. Here univalent B's are completely absent. The different shapes of the univalents in the various plants (rings or rods) also support the present interpretation that there are two kinds, standard B's and iso-chromosomes. This distinction, however, is not definitely established. There may be a single kind of B that behaves differently because of the different physiological conditions in different plants, some conditions in that case favouring a non-homologous pairing (or sticking together of chromosome parts) that simulates iso-chromosome behaviour and others not. FERNANDES (1946) found a considerable degree of non-homologous pairing in a heterochromatic supernumerary chromosome in *Narcissus*.

*Very small B-chromosomes.* — In two plants of the original sample there was found, besides the normal 5 bivalents, a very small univalent

chromosome (Figs. 25—27). Another plant was found to have a probably even smaller univalent besides $5_{II}$ and 3 of the ordinary B's. This extremely small chromosome was also observed in the progeny of this plant, so it can obviously maintain itself from one generation to another.



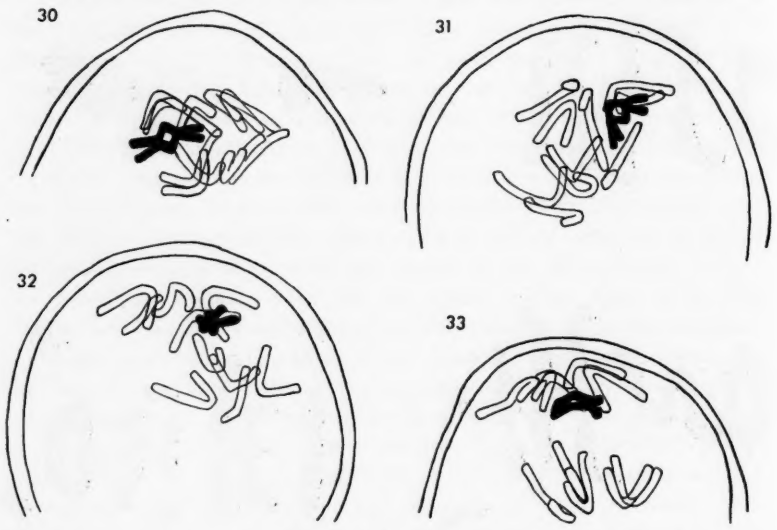

Figs. 25—27, meiosis in plants with B-chromosomes of the very small type. — Fig. 25, first metaphase. — Figs. 26—27, second metaphases from two different p.m.c's, in Fig. 26 delayed congression of the B.
Figs. 28—29, first metaphase in a plant with four B-chromosomes, at least two of which are iso-chromosomes. — Fig. 28, $4_{II} + 2_{I} + 1\ B_{IV}$. — Fig. 29, $5_{II} + 1\ B_{II} + 2$ B ring univalents. — × 3510.

These small chromosomes seem to be eliminated from the root tips, as pointed out above. At the first division they lie like ordinary univalents outside the metaphase group, and at the second division they manifest irregularities of congression on the plate (Fig. 26) and probably also of division. Hence they probably have an aberrant centromere.

Very small extra chromosomes are also known in maize, where they are called the F-type (RANDOLPH, 1941, p. 627).

## 6. FIRST POLLEN GRAIN MITOSIS.

Irregularities of behaviour at the first pollen mitosis are known in the case of the accessory chromosomes of *Secale cereale* (MÜNTZING, 1946 a) and *Sorghum purpureo-sericeum* (DARLINGTON and THOMAS, 1941). In both cases the two daughter halves of the B-chromosome fail to separate and are included in the generative nucleus, thus increasing their frequency in the germ-track. In the *Sorghum* case, however, the process is complicated by the occurrence of supernumerary mitoses.



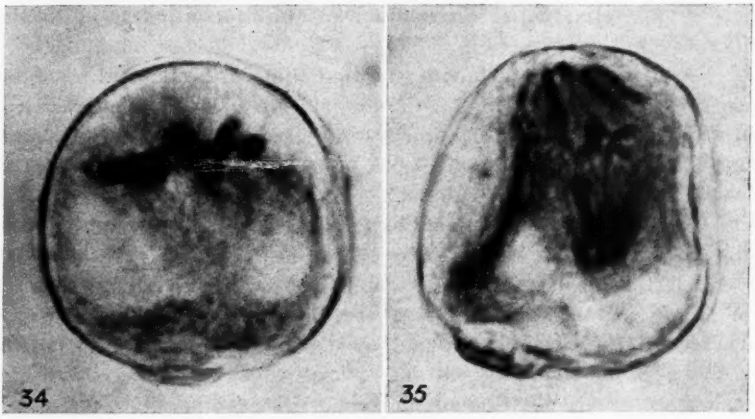

Figs. 30—33, anaphase of the first pollen mitosis, showing non-disjunction of the daughter B-chromosomes. — × 2150.

DARLINGTON and THOMAS suggest that the non-disjunction in *Sorghum* is due to an aberrant centromere, a rather likely hypothesis. In the rye case MÜNTZING has demonstrated that the centromere divides normally, but the two chromosomes remain associated in another chromosome region close to the centromere.

In *Anthoxanthum* could also be demonstrated a regular non-disjunction of the two halves of the B-chromosome to the generative nucleus. And my studies also clearly showed that the mechanism is quite the same at that found by MÜNTZING in rye.

At early anaphase we very often find in the B-chromosome plants the quite characteristic chromosome configurations depicted in Figs.

30—33. The B-chromosome is lagging between the two separating anaphase groups of normal chromosomes. It has a characteristic appearance with a central loop and four chromatid arms attached to it. The central loop is kept open by the attractions of the two daughter centromeres to the two opposite poles of the spindle. The B has a strong resemblance to a bivalent at the first metaphase of meiosis with a localized chiasma on each side of the centromere. This configuration demonstrates that the centromeres of the B's divide normally, but the two daughter chromosomes cannot separate because they are attached to one another at one point on each side of the centromere. The un-




Figs. 34—35, photographs of the first pollen mitosis. — Fig. 34, a metaphase in side view showing the asymmetric position of the mitotic figure. — Fig. 35, first anaphase showing non-disjunction of daughter B-chromosomes. — × 2080.

divided B-configuration is later included in that anaphase group which is lying close to the wall. (The mitotic spindle has a very asymmetric position in the pollen grain, with one pole close to the wall and the other in the central part of the grain.) This peripheral group is transformed into the generative nucleus and the central one becomes the vegetative nucleus.

The asymmetrical position of the mitotic figure is illustrated by the photograph Fig. 34, showing a metaphase in side view, and the characteristic lagging B-chromosome is given in the photograph Fig. 35. Here the central loop can be seen to be attenuated by the pull of the centromeres.

In other respects the pollen mitoses in these plants usually proceed normally. Normal mitoses were found in 1 plant with 4 B's, 3 plants with 2 B's and 2 plants with 1 B. In a single plant with 1 B, however, there was found lacking differentiation of vegetative and generative nuclei, combined with supernumerary mitoses. As development was normal in the other plants, it is reasonable to suppose that the B was not the chief cause of the abnormality; but it might, of course, have contributed to the effect.

The process outlined above contains two different factors deserving consideration from a mechanical point of view. Firstly, there is the attachment of the daughter chromosomes to one another, and secondly, there is the preferential distribution of the B configuration to the generative nucleus.

Considering the attachment, the first thing to be pointed out is its localized position. It is limited to one point on each side of the centromere. Thus, it cannot be due to a general stickiness of the chromosome, nor to a general delay of the lapse of chromatid attraction. This is clear from the observation that the rest of the chromosome arms are free from one another and often point in different directions. The same conclusion was arrived at by MÜNTZING in his rye case.

As to this localization, two possibilities may be suggested, viz. *either* the factor responsible for it is a *specially* (chemically or otherwise structurally) *differentiated segment* localized at the point of attachment, *or* there is no such segment of special constitution here, but the localization of the point of attachment is a kind of *position effect* due to the fact that this region is lying at a certain distance from other regions of decisive importance, such as the centromere or the chromosome ends. (In the latter case the determination of the position of attachment would be determined in a way somewhat related to that for determining the position of localized chiasmata in bivalents.) Even in the latter case there is of course also a special constitutional differentiation of the B-chromosome from the other chromosomes, because the others undergo no non-disjunction, but this special differentiation is then not localized at the points of attachment.

At present we are not in a position to distinguish between these two possibilities. Anyhow, in both cases another factor must also be of decisive importance, viz. a special physiological condition only found during the first pollen mitosis, as that is the only stage in the life cycle at which the effect is manifested. Thus the effect must be the result of an *interaction* between this special physiological condition in the

cell and some special constitutional property of the B-chromosome, whether this is localized in a special region or not.

MÜNTZING (1946 a), from his studies of the same process of non-disjunction in rye, also concluded that it resulted from such an interaction between a special chemical constitution in the fragment and the environment present in the pollen grains (l. c., p. 113). He suggested that there were special, probably heterochromatic segments close to the centromeres which caused the non-disjunction by their delayed reproduction, and also discussed the possibilities that it might be due to a delay of the lapse of chromatid attraction or some kind of stickiness.

I think that it will be impossible to determine at the present stage whether this localized chromatid attachment is due to a delayed reproduction or to some kind of localized stickiness or chromatid attraction.

The regular distribution of the B-configuration to the generative nucleus obviously suggests that the mitotic apparatus must be mechanically asymmetrical in one way or another. But the first pollen mitosis is, indeed, already known as a very asymmetrical mitosis. Not only are the two nuclei to which it gives rise very different from the very beginning, but the asymmetry is also often manifested in the shape of the spindle already at metaphase (GEITLER, 1935, p. 370). One pole is in contact with the cell-wall and is blunt, and the other is in the central part of the grain and is more pointed. I have found an asymmetry of the spindle of this type in the present material as well. The mitotic figure, here, is also asymmetrical in such manner that the metaphase plate is not lying half-way between the two poles but is closer to the generative pole. At the anaphase the generative chromosome group moves only a short distance and the vegetative one a longer distance. Undoubtedly the asymmetry must be an important factor in this mechanism of directed non-disjunction, even if the details of the process are not yet clear.

*Delayed pollen mitosis by B-chromosomes.* — It has already been found by DARLINGTON and THOMAS (1941) and MÜNTZING (1946) that pollen grains having accessory chromosomes divide later than the normal pollen grains in the same anther. I could demonstrate the existence of the same phenomenon in my material.

This phenomenon was studied in two plants with $2n = 10 + 1$ B and in one plant with $2n = 10 + 4$ B. The time of development of the anther was estimated (as was done by the authors cited) by counting the frequency of binucleate pollen.

The 1 B plants had, as expected, two kinds of pollen: $n = 5$ and $n = 5 + B$, the 4 B plant had in most cases $5 + 2$ B, but also $5 + 1$ B (from a 3 to 1 distribution at first anaphase) and in two cases only $n = 5$.

TABLE 1. *The correlation between the number of B-chromosomes at the first pollen mitosis and the average number of nuclei per pollen grain in the anther.*

| Chromosome number of plant | Total grains | Number of B's = $y$ | | | Total B's | Average of B's per mitosis | Average of nuclei per grain = $x$ | Number of grains for det. of $x$ |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | | | | |
| 10 + 1B | 4 | — | 4 | — | 4 | 1,000 | 1,940 | 116 |
| | 4 | — | 4 | — | 4 | 1,000 | 1,848 | 112 |
| | 29 | 6 | 23 | — | 23 | 0,793 | 1,690 | 87 |
| | 16 | 4 | 12 | — | 12 | 0,750 | 1,598 | 112 |
| | 24 | 9 | 15 | — | 15 | 0,625 | 1,560 | 91 |
| | 32 | 11 | 21 | — | 21 | 0,656 | 1,507 | 75 |
| 10 + 4B | 30 | — | — | 30 | 60 | 2,000 | 1,634 | 205 |
| | 29 | — | 1 | 28 | 57 | 1,966 | 1,453 | 95 |
| | 40 | 1 | 4 | 35 | 74 | 1,850 | 1,423 | 111 |
| | 31 | 1 | 30 | — | 30 | 0,968 | 1,039 | 206 |

Each line represents an anther.

The observations are given in Table 1. The material was statistically tested by the method of analysis of co-variance (BONNIER and TEDIN, 1940; MATHER, 1943). As the $y$-variate I used the number of B's of the individual pollen mitosis. This was correlated to the mean number of nuclei in the pollen grains of the anther ($= x$). The effect investigated was isolated as the joint regression for the two classes 1 B and 4 B. The analysis of variance of Table 1 is as follows:

| *Item* | *Sum of squares* | *N* | *Mean square* | *Probability* |
|---|---|---|---|---|
| 1. Between classes (1 B and 4 B) | 56,39 | 1 | — | — |
| 2. Between anthers within classes | 23,41 | 8 | — | — |
| 2 a. Joint regression ..... | 20,44 | 1 | 20,44 | < 0,001 |
| 2 b. Variation not due to joint regression ..... | 2,97 | 7 | 0,42 | 0,01—0,001 |
| 3. Within anthers ..... | 29,64 | 229 | 0,13 | — |
| 4. Total ..... | 109,43 | 238 | — | — |

The error to which the joint regression should be compared is the item 2 b. This item contains, besides variation from random sampling, also such variation as differences between the regressions of the two classes and deviations from linearity of the regressions. It is *a priori* extremely likely that both these kinds of variation exist in such a material, but they are not very interesting in the present connection, so no effort has been made to analyse them.

Anyhow, it is clear enough that there is a regression of the number of B-chromosomes in the mitoses on the frequency of binucleate pollen in the anthers, which demonstrates that pollen grains with B-chromosomes develop more slowly than such without.

The observations on the pollen mitoses do not suggest that there would be a more pronounced elimination of pollen grains having B-chromosomes. But, of course, the delayed division of B-pollen may be the expression of a lowered vigour, which may be manifested also later, e. g., at pollen tube growth. It is not known whether the reduced pollen fertility observed in B-plants affects B-pollen more strongly than normal pollen.

## IV. INHERITANCE OF B-CHROMOSOMES.

The inheritance of the B-chromosomes has been studied on progeny after *open pollination* of the original plants. The results should be supplemented by studies of controlled crosses, but the present observations also allow of some conclusions. The data are in agreement with the cytological observation of the non-disjunction of B's at the first pollen mitosis and they also demonstrate that *there is no such non-disjunction on the female side*; the effect is restricted to the pollen.

When the results submitted below are being considered it should be borne in mind that the number of individuals in the population containing the mother plants was relatively small, and that these plants did not flower all at the same time, and also that, because of the spatial conditions in the field, the different plants must have had variable possibilities for fertilizing certain other plants. Consequently, it is not justifiable to believe that the population of pollen grains which fertilized these studied mother plants was the same in all cases. Some mothers may have been fertilized chiefly by fathers with B-chromosomes, others by fathers without.

The results are summarized in Table 2, which is based on counts

TABLE 2. *Inheritance of B-chromosomes after open pollination.*

| Number of B's of mother | Number of B's of progeny | | | | | Number of plants per progeny |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| 0 | 7 | | 4 | | | 11 |
| 0 | 4 | | 3 | | 1 | 8 |
| 0 | 1 | | | | | 1 |
| 0 | 5 | | 2 | | | 7 |
| 0 | 4 | 1 | 3 | | | 8 |
| 0 | 3 | | 2 | | | 5 |
| 0 | 4 | | | | | 4 |
| 0 | 3 | | | | | 3 |
| 1 | 62 | 23 | 2 | | | 87 |
| 1—2[1] | 19 | 17 | | 1 | | 37 |
| 3 | | 14 | 9 | 1 | | 24 |
| 4 | | | 1 | | 2 | 3 |
| Total for 0 B mothers | 31 | 1 | 14 | | 1 | |
| Total for B mothers | 81 | 54 | 12 | 2 | 2 | |

[1] A chimaera.

of root-tip mitoses of plants the mothers of which were chromosome-counted at meiosis. The progenies from mothers without B-chromosomes must have received their B's from the pollen parent. The B-chromosomes are present in even numbers, 2 or 4, as expected from the cytological observations on the pollen mitosis. The exceptional plant with a single B may be due to a change in the chromosome number during the development of the plant, as such changes have been found to occur sometimes, or it may also indicate that the mechanism of non-disjunction in the pollen mitosis may sometimes fail.

Relatively big progenies were raised from three mothers having from 1 to 3 B's, and a small one from a plant having 4 B's. The three first mentioned progenies must have originated after pollination with pollen having only rarely B-chromosomes. This follows from a combined consideration of *firstly* the behaviour of B's in the pollen (known from the progeny of 0 B-mothers and from cytological studies) and *secondly* the chromosome numbers in these progenies. Thus the progeny after the 1 B mother consist nearly exclusively of 0 B and 1 B

plants. If the pollen had often contained B-chromosomes, we should often have had higher chromosome numbers. This should also have been the case if such a non-disjunction of B's occurred in the embryo-sacs too. Consequently, we can conclude that there is no such non-disjunction on the female side. The table indicates that there has also been a few cases in these progenies where the pollen has transferred B-chromosomes; at least this is a very probable suggestion.

The progenies of the 1 B- and 3 B-mothers should have given a 1 : 1 segregation into respectively 0 and 1 B, and 1 and 2 B. There is however in these cases a conspicuous deficit in the class with the higher number of B's. As the B's are not eliminated during the meiosis (seen in p.m.c's), the most probable explanation is a reduced viability of either embryo-sacs or embryos containing B-chromosomes. It should be mentioned that the seeds were stored from 1942 to 1946 before they were germinated, and the germination of these seed-samples was in many cases far from good. There may have been a higher frequency of B-individuals among the non-germinating seeds. (An alternative suggestion would be that the B's may partly be eliminated in the female meiosis although they are not during the corresponding male division.)

In the progeny of the 4 B-mother I got only 3 plants, presumably because of poor germination (there was, however, no exact record made of germination percentage). The two plants with four B's have most probably received two of their B's from the pollen parent.

(The reason there are so few plants in the progenies of 0 B-mothers is chiefly that it was thought better to have a few plants in many progenies than to have many plants in a few progenies.)

## V. PHENOTYPIC EFFECTS OF B-CHROMOSOMES.

There have not been observed any specific morphological features by which the B-chromosome plants differ from the others. It would, however, be very difficult to recognize such features, because the *Anthoxanthum* population in which they exist is so highly variable even without the influence of the B's (0 B plants). Such a high variability is also characteristic of the other *Anthoxanthum* populations I have studied, and hence it need not be connected in any way with the presence of B-chromosomes.

In general vigour as well as in pollen fertility the B plants show a slight reduction as compared with normal plants.

## 1. POLLEN FERTILITY.

The frequency of morphologically poor pollen was counted in pollen slides with the pollen mounted in a mixture of equal parts of acetocarmine and glycerine (a technique often used at this Institute).

TABLE 3. *Influence of B-chromosomes on pollen fertility.*

| Number of B's | Percentage of poor pollen 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | n | Mean % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 61 | 8 | 3 | 1 | 1 | | | | | | | 74 | 7,84 |
| 1 | 24 | 7 | 5 | 2 | 1 | 1 | | | | | 1 | 41 | 15,00 |
| 2 | 12 | 2 | 2 | 1 | | | | | | | | 17 | 10,29 |
| 3 | | | | 2 | | | | | | | | 2 | (35,00) |
| 4 | | | 1 | 1 | | | | | | | | 2 | (30,00) |

Total number of plants 136.

In each slide (with very few exceptions) exactly 200 grains (poor + good) were counted and the number of poor grains was recorded. The plants studied were from the same progenies as used above to observe the inheritance of the B's. The results are given in Table 3. For testing the results, the $\chi^2$ method was applied to the data after reducing them in the following way.

| | Poor pollen | |
|---|---|---|
| | $< 10\ \%$ | $> 10\ \%$ |
| Without B's ..... | 61 | 13 |
| With B's ..... | 36 | 26 |

$\chi^2 = 9{,}794$; $(0{,}01 > P > 0{,}001)$

Thus, there are fairly good reasons to conclude that the B-chromosomes cause a reduced pollen fertility. In Table 3 there is also a difference between plants with odd and even numbers of B-chromosomes, those with an odd number have a more reduced fertility, but the material is not sufficient to make this difference certain.

## 2. GENERAL VIGOUR.

The plants were classified with regard to general vigour on an arbitrary scale from 1 to 10 by inspection. This procedure was highly facilitated by the fact that the plants were cultivated in readily movable pots, so that it was possible to put any plant in a position between any two other plants to compare it closely with them in order to test whether it might be considered intermediate in vigour.

TABLE 4. *Earliness, vigour and B-chromosomes.*

| Degrees of earliness (1 = latest, 13 = earliest) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| 0;1 | 0;3 | 1;3 | 1;3 | 1;3 | 0;3 | 1;2 | 0;3 | 0;3 | 0;3 | 1;2 | 0;3 | 0;3 |
| 0;2 | 2;3 | 0;3 | 1;3 | 1;3 | 0;3 | 0;3 | 0;3 | 0;3 | 1;3 | 1;4 | 1;3 | 0;4 |
| 1;3 | 0;4 | 1;3 | 0;3 | 0;3 | 0;3 | 0;3 | 0;3 | 1;4 | 0;3 | 0;4 | 0;3 | 0;5 |
| 2;3 | 0;4 | 1;4 | 0;3 | 0;3 | 0;3 | 0;3 | 0;3 | 0;4 | 1;4 | 1;4 | 0;6 | 0;5 |
| 0;3 | 0;6 | 2;4 | 0;4 | 0;3 | 0;4 | 0;4 | 1;4 | 1;5 | 1;4 | 1;4 | 0;6 | 4;10 |
| 0;3 | 0;6 | 1;5 | 0;4 | 0;4 | 0;4 | 1;4 | 1;4 | 1;5 | 1;4 | 1;5 | 0;6 | |
| 1;4 | 1;6 | 0;5 | 0;5 | 1;4 | 0;4 | 0;4 | 0;4 | 0;5 | 0;4 | 0;6 | 1;7 | |
| 1;4 | 2;7 | 0;5 | 0;5 | 0;4 | 1;4 | 0;4 | 1;4 | 0;6 | 0;5 | 1;6 | 0;7 | |
| 1;4 | | 1;6 | 0;5 | 1;5 | 1;5 | 0;5 | 0;5 | 2;6 | 1;5 | 0;7 | | |
| 0;6 | | 0;6 | 0;5 | 0;6 | 1;5 | 1;5 | 2;5 | 2;7 | 1;5 | 0;7 | | |
| 2;6 | | 0;6 | 0;6 | 0;6 | 0;6 | 0;5 | 1;5 | 2;7 | 0;5 | | | |
| 0;6 | | | 0;6 | 0;6 | 1;6 | 0;7 | 0;5 | 1;7 | 1;5 | | | |
| 2;7 | | | 1;6 | | 0;6 | 0;7 | 1;6 | 0;7 | 0;6 | | | |
| | | | 1;7 | | 1;7 | | 2;6 | 3;8 | 3;6 | | | |
| | | | 0;9 | | 2;7 | | 0;6 | 4;8 | 0;6 | | | |
| | | | | | 0;8 | | 0;7 | 0;9 | | | | |
| | | | | | | | 1;7 | 0;9 | | | | |
| | | | | | | | 1;7 | | | | | |
| | | | | | | | 0;7 | | | | | |
| | | | | | | | 1;7 | | | | | |
| | | | | | | | 1;8 | | | | | |
| | | | | | | | 0;9 | | | | | |

1 square = 1 plant.
Left: number of B's.
Right: degree of vigour.
(1 = strongest,
10 = weakest).

The population studied is, however, extremely variable in earliness. Some plants have ripe seeds at a time when others have not yet started to flower. Obviously it was impossible to compare the vigour of plants so different in their stage of development. For this reason a »two-dimensional» sorting of the plants was applied. They were first sorted in an »east-western direction» by moving the pots into rows of similar earliness, as many rows as could be distinguished (= 13). Afterwards they were sorted inside these rows in a »north-southern direction» according to vigour. The most vigorous plant was put at one end of

each row and the least vigorous at the other, the rest being placed in various intermediate positions according to their appearance. Thus, the difficulties connected with the variation in earliness were overcome by classifying the vigour within classes of earliness. The plants were given numbers on an arbitrary scale, plants appearing to have the same vigour in a row being given the same number, an endeavour being made at the same time to employ numbers having as similar a value as possible in different groups of earliness.

The result of this classification is given in Table 4. Each square represents a plant and the columns correspond to the rows of varying earliness. For each plant is given the number of B-chromosomes (left) as well as the degree of vigour (right).

The effect of different number of B's on the vigour was statistically tested by analysis of covariance as the joint regression within earliness groups.

*Analysis of variance of vigour in Table 4.*

| *Item* | *Sum of squares* | *N* | *Mean square* | *Probability* |
|---|---|---|---|---|
| 1. Between groups of earliness | 54,11 | 12 | — | — |
| 2. Between groups of B's within groups of earliness ..... | 73,87 | 22 | 3,36 | Insignificant |
| 2 a. Joint regression ..... | 20,16 | 1 | 20,16 | 0,01—0,001 |
| 2 b. Differences between individual regressions ... | 44,17 | 12 | 3,68 | Insignificant |
| 2 c. Deviation from linearity | 9,54 | 9 | 1,06 | Insignificant |
| 3. Within groups of B's within groups of earliness ..... | 335,59 | 130 | 2,58 | — |
| 4. Total ..... | 463,58 | 164 | — | — |

The result strongly indicates that the vigour of the plants is reduced by increase in the number of B-chromosomes.

The material of Table 4 might also be used to study a correlation between earliness and number of B-chromosomes within classes of vigour. However, such a procedure is a little dubious, as because of the nature of the material it cannot be claimed that the classes of vigour given the same number really correspond when they are within different earliness groups. Anyhow, an analysis of variance of this type was performed on Table 4 and it failed to give any clear evidence of a correlation.

## VI. DISCUSSION.

Supernumerary chromosomes or chromosome fragments have been found in many different species. Undoubtedly they are of different nature in different cases. The simplest cases are those in which the supernumeraries are only duplications of normal chromosomes or chromosome parts. Such arise now and then in most organisms and are soon eliminated because of the genetic unbalance they cause.

Of more interest are those cases in which the extra chromosomes are not homologous with members of the normal complement, and thus cannot be newly formed from that. They have the ability to maintain themselves in the populations by special means (which in some cases are largely unknown) and obviously they must have done so for a very long time. Such chromosomes are known from *Zea mays* (e. g., RANDOLPH, 1941), *Sorghum purpureo-sericeum* (DARLINGTON and THOMAS, 1941), *Secale cereale* (MÜNTZING, 1943, 1944, 1945, 1946 a), *Crepis syriaca* (CAMERON, 1934), *Narcissus juncifolius* and *N. Bulbocodium* (FERNANDES, 1939, 1943, 1946), and *Godetia nutans* (HÅKANSSON, 1945). Probably many other less investigated cases of »extra fragments» also belong here; a list of »supernumerary fragment chromosomes» is given by DARLINGTON (1937, p. 145).

A discussion of such cases is given by HÅKANSSON (1945) in connection with his results from *Godetia.* Characteristic features of them are: (1) Absence of pairing with the normal chromosomes, (2) genetical inertness or subinertness, (3) they are often heterochromatic, (4) »defective» centromeres, causing change in number at mitosis, (5) frequently bad pairing at meiosis, (6) their ability to maintain themselves in the populations. Not all of these features, however, are present in all the cases; thus some cases are not heterochromatic (*Godetia* and *Secale*) and some do not have defective centromeres (especially *Secale*).

Undoubtedly the B-chromosomes of *Anthoxanthum* also belong here. They are not homologous with the normal chromosomes, they are subinert and heterochromatic, they maintain themselves in the population. Their centromeres, however, are but little defective (they may be more defective in the very small type), and they show a surprisingly good pairing with one another at meiosis. In the *Crepis syriaca* case CAMERON also found a very good pairing (1934, p. 267).

From the fact that the extra chromosomes remained in the populations it was concluded by DARLINGTON and UPCOTT (1941) and DARLINGTON and THOMAS (1941) that they were useful to the plants.

If they had not been useful, they would have been eliminated by natural selection.

Another suggestion concerning their function was made by ÖSTERGREN (1945), who suggested that they could quite well remain in the populations without being useful in any way to the plants. The decisive factor for the selection of them would rather be whether they were *useful to themselves*. They are probably in many cases leading an exclusively *parasitic existence* in the plants. It is known from MÜNTZING's observations (1945) that in *Secale* they performed a directed non-disjunction to the generative nucleus during the first pollen mitoses, thus increasing their frequency in the germ-track. This mechanism would cause natural selection to work on them in a way quite different from that on the normal chromosomes. While the normal chromosomes can increase only by being useful, the accessory chromosomes can increase quite irrespective of this by their special mechanism of multiplication. Although, of course, such a mechanism is different in its nature from the spread of parasites by infection, it will in practical results be something closely similar to infection. Accessory chromosomes with a more efficient mechanism of accumulation will be selected, and hence their evolution will tend to perfect this mechanism, and to decrease the factors causing elimination. As these mechanisms may be influenced by genes (e. g., those controlling the course of the first pollen mitosis), the accessory chromosomes will tend to accumulate a system of genes favouring their accumulation. The normal complement, on the other hand, may tend to accumulate genes favouring the elimination of B-chromosomes, so there may result an antagonism similar to that between a parasite and its host. They should also evolve to damage the host plant as little as possible and thus tend to become »inert» or »subinert». (For details I must refer to the original paper.)

The most important feature of such accessory chromosomes is their mechanisms of increasing in number. Theoretically, the same purpose may doubtless be gained in many different ways; but in all cases they must behave differently from the normal chromosomes at some stage or other in their life cycle. In *Anthoxanthum*, as well as in *Secale*, the differential mechanism consisted in a special behaviour of certain chromosome regions close to the centromere, where the daughter chromosomes attach themselves to one another. In *Secale* this mechanism functioned equally well in the female gametophyte as in the first pollen mitosis. In *Anthoxanthum*, however, it functioned only in the pollen. The *Anthoxanthum* case also differs from *Secale* in the absence of B-chro-

mosome elimination at meiosis, caused by the fact that here these chromosomes refuse to divide at the first division and divide regularly at the second. This latter mechanism most probably indicates that they have a centromere different from the normal chromosomes, as normal univalents divide at the first division. (An alternative suggestion might be that, here also, the same mechanism of non-disjunction functioned as is known from the pollen grains. It would be very difficult to recognize such a mechanism here because of the strong chromosome contraction at meiosis.)

In *Sorghum*, too, there is such a non-disjunction to the generative nucleus in the pollen mitosis. This is regarded by DARLINGTON and THOMAS as due to an aberrant centromere. Thus, the mechanism is thought to be different here from that later found in *Secale*, a suggestion that is of course quite possible. Here the chromosomes are also eliminated from the somatic tissue by the same centromere »defect», a mechanism which is useful to the B-chromosomes, as it will reduce the damage to the plant. In *Sorghum*, as in *Anthoxanthum*, there is no elimination of B-chromosomes at meiosis.

The maize data of RANDOLPH (1941) also strongly suggest that in this case, too, there may be some similar mechanism of non-disjunction in the pollen (l. c., pp. 618—619). Here, just as in *Anthoxanthum*, the special mechanism seems to work only on the male side.

In *Crepis syriaca* CAMERON also reports a clear tendency of the extra chromosomes to increase in number in the progeny. To explain this he assumes that these chromosomes may be favourable in one way or another and that consequently they may be selected in gametes or zygotes. Seen in connection with the present results, it seems, however, more likely that they have a special mechanism to produce an increase in number irrespective of selection.

Concerning *Godetia nutans*, HÅKANSSON also stresses the absence in the progenies of any tendency of the accessory chromosomes to decrease. Here, as in *Anthoxanthum*, the B-univalents do not divide at the first meiotic division, and they are practically not eliminated during meiosis. Whether they also have a mechanism leading to an actual increase of them is not known.

Some cases have been reported in which the B-chromosomes seem to have »weak» centromeres, causing them to behave irregularly at mitosis (e. g., *Tulipa galatica* and also *Zea mays*; DARLINGTON and UPCOTT, 1941, p. 279). I think that the abnormal behaviour during the mitoses is due to the same differentiation of the centromere of the B-

chromosomes as enables them to increase their frequency by non-disjunction at some suitable moment in their life cycle. The risk of mitotic elimination is the price which these chromosomes must pay for the favour of being able to increase mechanically in number. Although the centromere may be »weak» from a mechanical point of view, this specialization may in reality be a *strength* of the B-chromosome from a genetical point of view. An extreme case of such variation is described in *Ranunculus acris* by LANGLET (1927), where the number of extra chromosomes varied in a single root tip between 2 and 10, with an average of about 6. Such a variation during the development of the individual might in some cases even be a sufficient means of increasing the frequency of extra chromosomes, if the changes in number occur in an opportunistic way so that gains are more common than losses for the germ-track. It may be of some significance that in *Secale*, where the accumulation mechanism is not the centromere but another chromosome region, the »fragments» show a great stability at mitosis.

According to my interpretation, the equilibrium of such chromosomes in the populations would consist in a balance between a mechanical increase in number and a selective decrease caused by the harmful effects on vigour and fertility which they cause.

To test this hypothesis it will be very valuable to have exact determinations of the efficiency of the accumulation mechanisms of the B's and also to have exact information concerning their phenotypic effects, data which as yet may be insufficient.

The only phenotypic effects actually observed from B-chromosomes are deleterious ones. In maize these chromosomes, when few, have no visible effects. In higher numbers (more than 10—15) they cause reduction of fertility and vigour (RANDOLPH, 1941). In rye, as well as in *Anthoxanthum*, these effects are demonstrable even in plants with a much lower number of accessory chromosomes (MÜNTZING, 1943; and the results above). In *Crepis syriaca* CAMERON (1934) reports reduced fertility and morphological abnormalities from the extra chromosomes. In *Sorghum* they cause reduced pollen fertility and a cytological abnormality called »morbid mitosis» (DARLINGTON and THOMAS, 1941).

The beneficial effects sometimes assumed (CAMERON, 1934; DARLINGTON and UPCOTT, 1941; DARLINGTON and THOMAS, 1941) are as yet exclusively hypothetical.

The strongest support for beneficial effects of the B-chromosomes in maize is furnished by the observations on their inheritance given by DARLINGTON and UPCOTT (l. c., p. 290). The strong loss of the B's

observed there certainly indicates that there must be a positive selection counteracting this loss, and in my paper of 1945 I, too, endorsed the possibility of their being useful (l. c., p. 163).

When studying the data of RANDOLPH (1941), however, we do not at all find such pronounced losses in the progenies of plants with few B-chromosomes. (Only progenies of plants with few B's will be important, because many B's will only very rarely be found in the same plant in populations.) They seem here to be transferred in practically constant frequency. This makes me believe that quite possibly the B's of maize are also parasitic. Now, the B-chromosomes are known to pass through chromosome structural changes rather often. Probably different breeding results can be obtained by employing different types of B's. RANDOLPH (1941, p. 613) reports that POWERS and DAHL had obtained breeding results quite different from his own with their material. I think such differences may be due to this cause. It should be observed that if various mutant B's are found to be eliminated, this is a fact of but little interest in the present connection.

In *Secale, Sorghum* and probably *Crepis* the accumulation mechanism seems to have a sufficient efficiency for my hypothesis. *Anthoxanthum* requires further investigation before any statements can be made on this point, but most probably this is the case here, too.

There is a point in my discussion of 1945 which I should like to improve. On pages 160—161 I suggested that if there arose valuable properties in an extra chromosome these would most probably, sooner or later, be »stolen» by the normal complement and thus lose their importance for the B-chromosome (see the original for details). However, this process will only occur if the B-chromosome has a weak mechanism of accumulation. If the B-chromosome possesses a strong ability to increase its frequency, the reverse process may follow. By the mechanical increase of the B-chromosomes the population will get an *overdosage* of the gene or chromosome segment in question. Consequently, selection will favour a decrease of dosage of this gene in the normal complement, and the plant may in this way become dependent on the B in order to get its need satisfied in this respect.

Something similar may also occur in other cases where the B-chromosomes do not contain any special favourable genotypic components, by simple adaptation of the population to the B-chromosomes. This is illustrated by Fig. 36. Curve I represents the relation in a population which has newly been infected by B-chromosomes. Obviously genes will be selected in the population which make B-chromo-

some plants more fertile and more vigorous than they were originally: the plant will tend to adapt itself to the B's. When the B's in this way turn less harmful they will increase in number, because it was

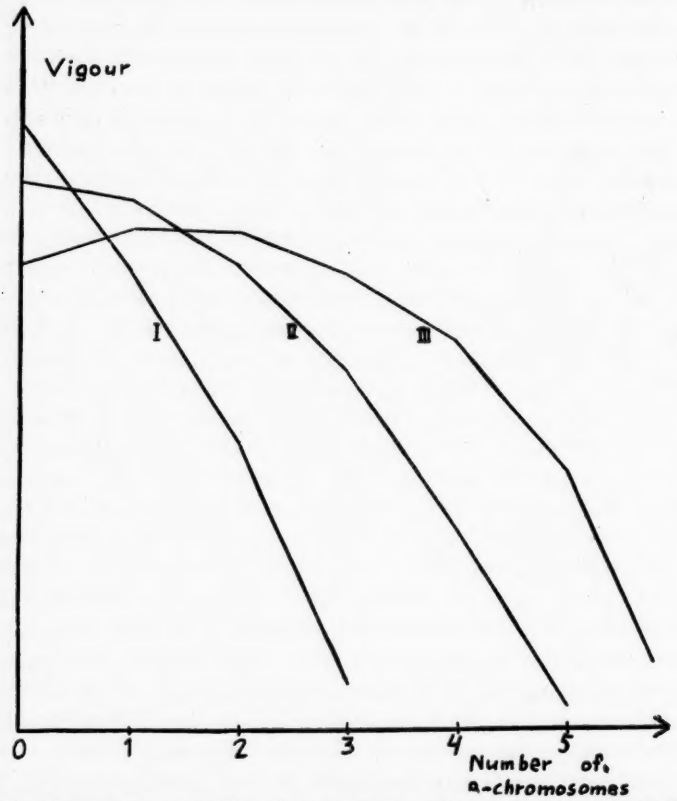



Fig. 36. Hypothetical evolution of a population which becomes infected by B-chromosomes. I = the effect before the population has changed, and II and III various degrees of adaptation of the plant to the presence of B-chromosomes.

their deleterious effects which previously prevented them from increasing. The curve has changed to II in Fig. 36. This will cause a need for further adaptation, and the curve may change to III, etc. As the gene balance in the original population without the B's was the optimal which natural selection could produce in the population, it is likely that the change in balance required for the adaptation to the B's will also cause the plants without B's to be less well adapted. Thus we could

get a situation where plants with a few B's were more successful than plants without B's; but it would be a mistake to conclude from this that the B's had improved the population by their presence.

LONGLEY (1938) had found that maize races with a high frequency of B-chromosomes had a lower frequency of heterochromatic knobs in the normal chromosomes. This may be caused by a mechanism of the type just outlined. The B-chromosomes may impose themselves on the population and, as they produce an overdosage of heterochromatin, the normal complement may have been forced to reduce its heterochromatin. Thus, it need not be concluded from such observations that the B's are useful.

It has been suggested by MATHER (1944) that the apparent inertness of accessory chromosomes is not due to a real absence of genic activity, but that it is caused by the fact that the various genic activities in the B-chromosomes together form a balanced system. This idea is supported by the fact that there seem to exist certain *standard types* of accessory chromosomes. Thus, the extra chromosomes of rye are closely similar to one another in the most various populations (MÜNTZING, 1945, pp. 471—472). LONGLEY (1938), who studied the B-chromosomes of maize from 14 races, found them with a single exception identical in appearance. As the B's retain their type in spite of the fact that aberrant extra chromosomes are very often formed, I consider that there must obviously exist a certain standard type that has a selective advantage. RANDOLPH (1941, pp. 628—629) got the impression that a B-chromosome fragment (called D) might have stronger deleterious effects than the B itself. All this favours the idea that they are not actually inert but that their genic activities together form a harmoniously balanced system somewhat similar to that of a whole set of normal chromosomes. This system, I think, would be selected in such a way that it favours, as far as possible, the accumulation mechanism of the B's and otherwise that it injures the plant as little as possible.

Nothing is known concerning the origin of such B-chromosomes. They seem, however, to be well differentiated from the normal chromosomes. RANDOLPH (1941) in his careful study failed to find any support for the idea that the B-chromosomes of maize could be directly derived from the normal chromosomes. Such extra chromosomes can quite well be very old. They may very well have existed in the ancestors of *Anthoxanthum aristatum* so far back as when these plants did not belong to the genus *Anthoxanthum*.

It is also possible that such parasitic chromosomes may spread

from one species to another by species hybridization followed by back-cross of the hybrid to that species which, previously, was free from parasitic chromosomes. This may be an important factor in Gramineae, where even very widely separated species may cross, such as maize and sugar cane (JANAKI-AMMAL, 1941).

Anyhow, such accessory chromosomes must have originated at some time. It is not likely that a successful B-chromosome can originate from a normal chromosome by a single change (mutation or structural re-arrangement); probably several mutations must have occurred simultaneously or nearly so. The probability per generation of such an occurrence is extremely small, but, in view of the fact that there have been millions of generations available in the past, the chance of such an occurrence will be much greater. When once they have originated they can easily evolve further and differentiate from one another, giving various other types of B-chromosomes, and also most probably they can extend their distribution by also infecting other species by species hybridization.

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## SUMMARY.

A population of *Anthoxanthum aristatum* has been found to contain a variable number of extra chromosomes besides the normal diploid chromosome complement of 10 chromosomes. These B-chromosomes are heterochromatic. They do not pair with chromosomes of the normal complement, but they show a very good pairing with one another. Univalent B-chromosomes are not eliminated at meiosis, differing in this respect from univalents of the normal chromosomes. At the first pollen mitosis the two daughter halves of the B-chromosomes remain attached to one another and are both included in the generative nucleus, thus increasing their frequency in the germ-track. The inheritance of B-chromosomes demonstrates that such a non-disjunction occurs only in the pollen but not in the embryo-sac. The B-chromosomes cause a delay of the development of the pollen grain and a slight reduction of pollen fertility and plant vigour. They are regarded as belonging to the category of parasitic chromosomes, and are discussed in connection with other similar cases.

## LITERATURE CITED.


1. BONNIER, G. and TEDIN, O. 1940. Biologisk variationsanalys. — Stockholm.
2. CALLAN, H. G. 1942. Heterochromatin in *Triton*. — Proc. Roy. Soc. London, B 130: 324—335.
3. CAMERON, D. R. 1934. The chromosomes and relationship of *Crepis syriaca* (BORNM.). — Univ. Cal. Publ. Agr. Sci. 6: 257—286.
4. COLEMAN, L. C. 1943. Chromosomal structure in the Acrididae with special reference to the x-chromosome. — Genetics 28: 1—8.
5. COUTINHO, A. X. P. 1939. Flora de Portugal. — Lisboa.
6. DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd ed. — London.
7. DARLINGTON, C. D. and LA COUR, L. 1938. Differential reactivity of the chromosomes. — Ann. of Bot. N. S. II: 615—625.
8. — and —. 1940. Nucleic acid starvation of chromosomes in *Trillium*. — Journ. of Gen. 40: 185—213.
9. — and —. 1941. The detection of inert genes. — Journ. of Heredity 32: 115—121.
10. DARLINGTON, C. D. and THOMAS, P. T. 1941. Morbid mitosis and the activity of inert chromosomes in *Sorghum*. — Proc. Roy. Soc. London, B 130: 127—150.
11. DARLINGTON, C. D. and UPCOTT, M. B. 1941. The activity of inert chromosomes in *Zea mays*. — Journ. of Gen. 41: 275—296.
12. FERNANDES, A. 1939. Sur le comportement d'un chromosome surnuméraire pendant la mitose. — Scientia Genetica I: 141—167.
13. — 1943. Sur l'origine des chromosomes surnuméraires hétérochromatiques chez *Narcissus Bulbocodium*. — Bol. Soc. Broteriana, 2 série XVII: 251—256.
14. — 1946. Sur le comportement des chromosomes surnuméraires hétérochromatiques pendant la méiose. I. Chromosomes longs hétérobrachiaux. — Bol. Soc. Broteriana, 2 série XX: 93—138.
15. GEITLER, L. 1935. Beobachtungen über die erste Teilung im Pollenkorn der Angiospermen. — Planta 24: 361—386.
16. — 1937. Die Analyse des Kernbaus und der Kernteilung der Wasserläufer *Gerris lateralis* und *Gerris lacustris* (Hemiptera, Heteroptera) und die Somadifferenzierung. — Zschr. f. Zellf. u. mikr. Anat. 26: 641—672.
17. — 1939. Das Heterochromatin der Geschlechtschromosomen bei Heteropteren. — Chromosoma I: 197—229.
18. — 1940. Temperaturbedingte Ausbildung von Spezialsegmenten an Chromosomenenden. — Chromosoma I: 554—561.
19. HEITZ, E. 1928. Das Heterochromatin der Moose. I. — Jahrb. f. wiss. Bot. 69: 762—818.
20. — 1932. Die Herkunft der Chromozentren. — Planta 18: 571—635.
21. HÅKANSSON, A. 1945. Überzählige Chromosomen in einer Rasse von *Godetia nutans* HIORTH. — Bot. Notiser 1945: 1—19.
22. JANAKI-AMMAL, E. K. 1941. Intergeneric hybrids of *Saccharum*. — Journ. of Gen. 41: 217—253.
23. KATTERMANN, G. 1931. Über die Bildung polyvalenter Chromosomenverbände bei einiger Gramineen. — Planta 12: 732—744.

24. KLINGSTEDT, H. 1941. Negative heterochromacy in Orthopteran chromosomes. — Mem. Soc. pro Fauna et Flora Fennica 17: 166—175.
25. KOLLER, P. C. 1938. The genetical and mechanical properties of the sex-chromosomes. II. The golden hamster. — Journ. of Gen. 36: 177—195.
26. LANGLET, O. F. J. 1927. Beiträge zur Zytologie der Ranunculazeen. — Sv. Bot. Tidskr. 21: 1—17.
27. LEVAN, A. 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII: 177—211.
28. LEVITSKY, G. A. 1931. The morphology of chromosomes. — Bull. Appl. Bot., Genet. and Plant-Breed. XXVII: 19—174.
29. LONGLEY, A. E. 1938. Chromosomes of maize from North American Indians. — Journ. of Agric. Res. 56: 177—195.
30. MATHER, K. 1943. Statistical analysis in biology. — London.
31. — 1944. The genetical activity of heterochromatin. — Proc. Roy. Soc. London, B 132: 308—332.
32. MÜNTZING, A. 1933. Apomictic and sexual seed formation in *Poa*. — Hereditas XVII: 131—154.
33. — 1943. Genetical effects of duplicated fragment chromosomes in rye. — Hereditas XXIX: 91—112.
34. — 1944. Cytological studies of extra fragment chromosomes in rye. I. Iso-fragments produced by misdivision. — Hereditas XXX: 213—248.
35. — 1945. Cytological studies of extra fragment chromosomes in rye. II. Transmission and multiplication of standard fragments and iso-fragments. — Hereditas XXXI: 457—477.
36. — 1946 a. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at the pollen mitosis. — Hereditas XXXII: 97—119.
37. — 1946 b. Different chromosome numbers in root tips and pollen mother cells in a sexual strain of *Poa alpina*. — Hereditas XXXII: 127—129.
38. PARTHASARATHY, N. 1939. Cytogenetical studies in Oryzeae and Phalarideae. III. Cytological studies in Phalarideae. — Ann. of Bot. N. S. III: 43—76.
39. RANDOLPH, L. F. 1941. Genetic characteristics of the B chromosomes in maize. — Journ. of Agric. Res. 56: 177—195.
40. WHITE, M. J. D. 1933. Tetraploid spermatocytes in a locust, *Schistocerca gregaria*. — Cytologia 5: 135—139.
41. — 1935. The effects of X-rays on mitosis in the spermatogonial divisions of *Locusta migratoria* L. — Proc. Roy. Soc. London, B 119: 61—84.
42. — 1940. The heteropycnosis of sex chromosomes and its interpretation in terms of spiral structure. — Journ. of Gen. 40: 67—82.
43. — 1945. Animal cytology and evolution. — Cambridge.
44. ÖSTERGREN, G. 1942. Chromosome numbers in *Anthoxanthum*. — Hereditas XXVIII: 242—243.
45. — 1945. Parasitic nature of extra fragment chromosomes. — Bot. Notiser 1945: 137—163.

# ABSTRACTS — KURZE MITTEILUNGEN

TORSTEN ROMANUS: Psoriasis in twins.

If a hereditary disease which can appear for the first time at different ages is discordant in monozygous twins, the cause may be that the disease has not had time to show itself in the other twin. When one has to decide whether there is discordance in psoriasis, it is therefore necessary to consider the observation period. In an investigation of 768 post-examined psoriasis cases (ROMANUS, 1945) three-fourths of the men developed the disease before the age of 26 and three-fourths of the women before the age of 19. If, therefore, one reckons with 30 years of age as a limit, it might be said that in most of the cases of psoriasis the disease has appeared at that age.

In the following table are collocated cases of psoriasis in monozygous twins, described in the literature.

| Author | Year | Sex | Age, years | Age at onset of psoriasis |
|---|---|---|---|---|
| | | *Concordance* | | |
| SIEMENS | 1924 | females | 32 | Since childhood |
| WEITZ | 1925 | males | 58 | No information |
| CLARK and STIBBENS | 1926 | » | 14 | 14; 14 |
| v. VERSCHUER | 1927 | females | 12 | No information |
| ZIELER | 1930 | — | — | » » |
| VOHWINKEL | 1932 | males | — | 8; 8 |
| WEBER | 1934 | females | 31 | 22; 26 |
| MAYR | 1938 | » | c. 34 | c. 20; c. 30 |
| KAMPEN | 1941 | » | 14 | 6; 6 |
| LIEBENAM | 1942 | » | 13 | 12 $^1/_2$; 13 |
| | | *Discordance* | | |
| GLATZEL | 1931 | males | 13 | c. 10 |
| DOLLMAN v. OYE | 1939 | females | 10 | c. 9 |
| MELSOM | 1945 | » | 18 | 8 |
| v. VERSCHUER | 1927 | males | 50 | No information |
| GLATZEL | 1931 | » | 38 | c. 28 |
| » | » | females | 33 | No information |
| ROMANUS | 1945 | » | 31 | 4 |
| » | the present case | » | 43 | 9 |

Of the cases in question 10 pairs of monozygous twins were concordant in regard to psoriasis, whereas 4 pairs (with the present case 5) were discordant. Further, discordance has been found for 3 pairs of monozygous twins, aged respectively 10, 13 and 18 years. In these cases, the observation period consequently is rather short.

In the present case (see pedigree) it is a question of two monozygous twin sisters (proband = P), born in 1902. At the time of the examination

they were 43 years of age. They bear a strong likeness which has often led to their being mistaken for each other. Their relatives and friends have mainly been able to distinguish them because of their different temperament. They were born at home and therefore it has not been possible to obtain any information as to the egg membranes.

In the last-born twin (III P) efflorescences of psoriasis appeared on the scalp and the elbows at the age of 9 years. Later on she had disseminated psoriasis punctata on the trunk and the upper extremities. No seasonal variations. She has never been without symptoms. No pregnancy. At the examination in February, 1946, she had a small number of round efflorescences on the scalp and the elbows, varying from $^1/_2$ to 1 cm. in diameter.

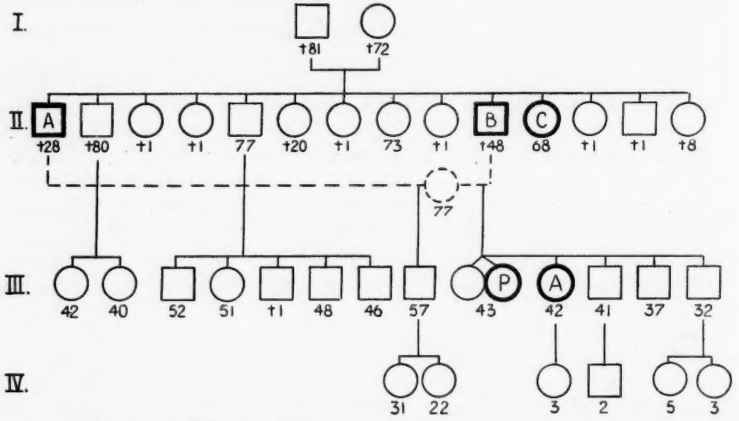


Fig. 1. Pedigree. — Square = man. Circle = woman. A, B, C and P = person affected by psoriasis. ⌒ = twins.

The twin sister has never had any skin affections and did not show any at the examination.

A paternal uncle (II A) who earlier was married to the proband's mother also suffered from psoriasis. According to the record (in 1883) from the Medical Department of the University Hospital at Uppsala where he was treated for psoriasis, the efflorescences appeared at the age of 17 on the left lower leg; later also the scalp, arms and trunk were affected. According to the record, his parents (I) had not had any skin disease; on the other hand, a brother (II B) and a sister (II C) had psoriasis. This sister has in 1946 given the information that her brother, i. e. the paternal uncle mentioned above, after having left the hospital only had few and slight symptoms of psoriasis until his death at the age of 28. It is stated that his son and two grandsons have never had any symptoms of psoriasis.

The proband's father (II B), it is reported, had psoriasis already as a child but only on elbows and legs. He emigrated to the U. S. A. in 1912, which is probably the reason there is no further information about the course of the disease. He died at the age of 48 years.

A paternal aunt (II C) of the proband, who has been examined by the author, has suffered from psoriasis from her childhood but only to a slight degree. She has had circinated eruptions on the trunk, and efflorescences on the elbows; the last 4 years, however, she has only had a few efflorescences on the right elbow, which was the case also at the examination in February, 1946. She has never been free from symptoms.

One of the proband's sisters (III A) has had psoriasis since she was 5 years old. At this age large confluent efflorescences appeared on the lower legs. Later on she had eruptions on the scalp, the trunk and the extremities. At the examination in March, 1946, she had efflorescences on the scalp, and scattered efflorescences on the trunk, elbows and legs. No seasonal variations. Improvement during pregnancy at 39 years of age. She has never been free from symptoms. She has a daughter, 3 years old, who up to the present has not been affected by psoriasis.

We have here a case of psoriasis where the disease has affected only one of two monozygous twin sisters. The cause of the discordance could hardly be too short an observation period, since the twin who suffers from psoriasis got her disease already as a child (9 years of age) and the age of the twins now is 43 years. It is scarcely probable that the twin who up to now has been free of symptoms should be affected by psoriasis later, although this possibility cannot be quite excluded.

There is, however, one more possibility of explaining discordance in monozygous twins. The character may be a genotypic asymmetry (DAHLBERG, 1926 and 1943). From this point of view the localization of the psoriasis-efflorescences is of interest. A paternal uncle had psoriasis only on the left lower leg and, later, on the trunk and arms. A paternal aunt had efflorescences only on the upper part of the body (trunk and arms) and during the last 4 years only on the right elbow. Then there is good reason to believe that in this case the discordance is genotypically determined, so that the gene has only a one-sided effect. In an investigation of psoriasis with asymmetrical localization the present author (ROMANUS, 1945) found that of 478 cases post-examined by him, 71 (14,9 %) had efflorescences with asymmetrical localization. In 8 of these cases the efflorescences were localized (since at least 20 years ago) only on one side of the body. This circumstance as also the cases of discordant psoriasis in monozygous twins speak in favour of the hypothesis that psoriasis can be a genotypic asymmetry, and are also a support of DAHLBERG's theory of genotypic asymmetries.

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Literature cited.

1. CLARK, T. J. and STIBBENS, F. H. 1926. Skin diseases in twins. — California and West. Med. V. 24, p. 777. (Ref. Zentralbl. f. Haut- u. Geschlechtskr. V. 21, p. 170.)
2. DAHLBERG, G. 1926. Twin births and twins from a hereditary point of view. — Diss. Stockholm.
3. — 1943. Genotypic asymmetries. — Proc. Soc. Edinburgh. V. 62, p. 20.

4. DOLLMAN v. OYE, W. 1939. Psoriasis universalis. — Zentralbl. f. Haut- u. Geschlechtskr. V. 63, p. 108.
5. GLATZEL, H. 1931. Beiträge zur Zwillingspathologie. — Ztschr. f. klin. Med. V. 116, p. 666.
6. v. KAMPEN, B. 1941. Beitrag zur Zwillingspathologie der Psoriasis. — Diss. Freiburg.
7. LIEBENAM, L. 1942. Konkordantes Vorkommen von Psoriasis vulgaris bei einem eineiigen Zwillingspaar. — Der Erbartzt, V. 10, p. 248.
8. MAYR, F. 1938. Zur Vererbung bei der Psoriasis vulgaris. — Dermat. Wchnschr. V. 106, p. 569.
9. MELSOM, R. 1945. Dermatological investigations on 22 pairs of identical twins. — Acta Dermat.-venereol. V. 25, p. 29.
10. ROMANUS, T. 1945. Psoriasis from a prognostic and hereditary point of view. — Acta Dermat-venereol. V. 26, Suppl. 12.
11. SIEMENS, H. W. 1924. Die Zwillingspathologie. — Berlin.
12. v. VERSCHUER, O. 1927. Die vererbungsbiologische Zwillingsforschung. — Ergebn. d. inn. Med. u. Kinderh. V. 31, p. 35.
13. VOHWINKEL, K. H. 1932. Beitrag zur Zwillingspathologie der Psoriasis. — Dermat. Wchnschr. V. 94, p. 340.
14. WEBER, F. P. 1934. Psoriasis arthropathica in a woman whose twin sister was similarly affected. — Proc. Roy. Soc. Med. V. 27, p. 589.
15. WEITZ, W. 1925. Studien an eineiigen Zwillingen. — Ztschr. f. klin. Med. V. 101, p. 115.
16. ZIELER, K. 1930. Ergebnisse der Untersuchungen über die Erblichkeit der Schuppenflechte. — Arch. f. Dermat. u. Syph. V. 160, p. 57.